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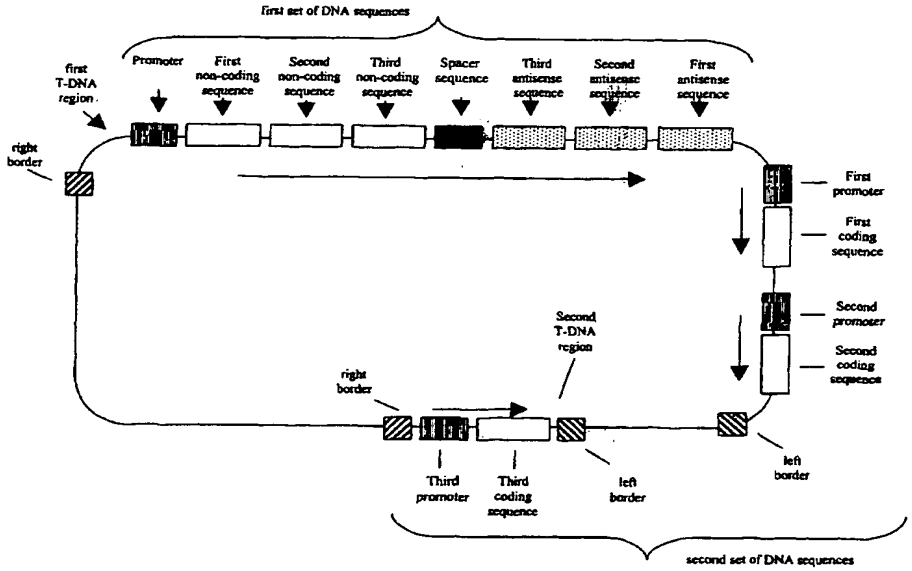
(54) Title: NUCLEIC ACID CONSTRUCTS AND METHODS FOR PRODUCING ALTERED SEED OIL COMPOSITIONS



(57) Abstract: The present invention is in the field of plant genetics and provides recombinant nucleic acid molecules, constructs, and other agents associated with the coordinate manipulation of multiple genes in the fatty acid synthesis pathway. In particular, the agents of the present invention are associated with the simultaneous enhanced expression of certain genes in the fatty acid synthesis pathway and suppressed expression of certain other genes in the same pathway. Also provided are plants incorporating such agents, and in particular plants incorporating such constructs where the plants exhibit altered seed oil compositions.

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NUCLEIC ACID CONSTRUCTS AND METHODS FOR PRODUCING ALTERED SEED OIL COMPOSITIONS

FIELD OF THE INVENTION

The present invention is directed to recombinant nucleic acid molecules, constructs, and other agents associated with the coordinate manipulation of multiple genes in the fatty acid synthesis pathway. In particular, the agents of the present invention are associated with the simultaneous enhanced expression of certain genes in the fatty acid synthesis pathway and suppressed expression of certain other genes in the same pathway. The present invention is also directed to plants incorporating such agents, and in particular to plants incorporating such constructs where the plants exhibit altered seed oil compositions.

BACKGROUND

Plant oils are used in a variety of applications. Novel vegetable oil compositions and improved approaches to obtain oil compositions, from biosynthetic or natural plant sources, are needed. Depending upon the intended oil use, various different fatty acid compositions are desired. Plants, especially species which synthesize large amounts of oils in seeds, are an important source of oils both for edible and industrial uses. Seed oils are composed almost entirely of triacylglycerols in which fatty acids are esterified to the three hydroxyl groups of glycerol.

Soybean oil typically contains about 16-20% saturated fatty acids: 13-16% palmitate and 3-4% stearate. *See generally* Gunstone *et al.*, *The Lipid Handbook*, Chapman & Hall, London (1994). Soybean oils have been modified by various breeding methods to create benefits for specific markets. However, a soybean oil that is broadly beneficial to major soybean oil users such as consumers of salad oil, cooking oil and frying oil, and industrial markets such as biodiesel and biolube markets, is not available. Prior soybean oils were either too expensive or lacked an important food quality property such as oxidative stability, good fried food flavor or saturated fat content, or an important biodiesel property such as appropriate nitric oxide emissions or cold tolerance or cold flow.

Higher plants synthesize fatty acids via a common metabolic pathway -- the fatty acid synthetase (FAS) pathway, which is located in the plastids. β-ketoacyl-ACP synthases are important rate-limiting enzymes in the FAS of plant cells and exist in several versions. β-ketoacyl-ACP synthase I catalyzes chain elongation to palmitoyl-ACP (C16:0), whereas β-

ketoacyl-ACP synthase II catalyzes chain elongation to stearoyl-ACP (C18:0). β-ketoacyl-ACP synthase IV is a variant of β-ketoacyl-ACP synthase II, and can also catalyze chain elongation to 18:0-ACP. In soybean, the major products of FAS are 16:0-ACP and 18:0-ACP. The desaturation of 18:0-ACP to form 18:1-ACP is catalyzed by a plastid-localized soluble delta-9 desaturase (also referred to as "stearoyl-ACP desaturase"). *See* Voelker *et al.*, 52 *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 335-61 (2001).

The products of the plastidial FAS and delta-9 desaturase, 16:0-ACP, 18:0-ACP, and 18:1-ACP, are hydrolyzed by specific thioesterases (FAT). Plant thioesterases can be classified into two gene families based on sequence homology and substrate preference. The first family, FATA, includes long chain acyl-ACP thioesterases having activity primarily on 18:1-ACP. Enzymes of the second family, FATB, commonly utilize 16:0-ACP (palmitoyl-ACP), 18:0-ACP (stearoyl-ACP), and 18:1-ACP (oleoyl-ACP). Such thioesterases have an important role in determining chain length during de novo fatty acid biosynthesis in plants, and thus these enzymes are useful in the provision of various modifications of fatty acyl compositions, particularly with respect to the relative proportions of various fatty acyl groups that are present in seed storage oils.

The products of the FATA and FATB reactions, the free fatty acids, leave the plastids and are converted to their respective acyl-CoA esters. Acyl-CoAs are substrates for the lipid-biosynthesis pathway (Kennedy Pathway), which is located in the endoplasmic reticulum (ER). This pathway is responsible for membrane lipid formation as well as the biosynthesis of triacylglycerols, which constitute the seed oil. In the ER there are additional membrane-bound desaturases, which can further desaturate 18:1 to polyunsaturated fatty acids. A delta-12 desaturase (FAD2) catalyzes the insertion of a double bond into 18:1, forming linoleic acid (18:2). A delta-15 desaturase (FAD3) catalyzes the insertion of a double bond into 18:2, forming linolenic acid (18:3).

Many complex biochemical pathways have now been manipulated genetically, usually by suppression or over-expression of single genes. Further exploitation of the potential for plant genetic manipulation will require the coordinate manipulation of multiple genes in a pathway. A number of approaches have been used to combine transgenes in one plant – including sexual crossing, retransformation, co-transformation, and the use of linked transgenes. A chimeric transgene with linked partial gene sequences can be used to

coordinately suppress numerous plant endogenous genes. Constructs modeled on viral polyproteins can be used to simultaneously introduce multiple coding genes into plant cells. For a review, *see* Halpin *et al.*, *Plant Mol. Biol.* 47:295-310 (2001).

Thus, a desired plant phenotype may require the expression of one or more genes and the concurrent reduction of expression of another gene or genes. Thus, there exists a need to simultaneously over-express one or more genes and suppress, or down-regulate, the expression of a another gene or genes in plants using a single transgenic construct.

SUMMARY OF THE INVENTION

The present invention provides a nucleic acid molecule or molecules, which when introduced into a cell or organism are capable of suppressing, at least partially reducing, reducing, substantially reducing, or effectively eliminating the expression of at least one or more endogenous *FAD2*, *FAD3*, or *FATB* RNAs while at the same time coexpressing, simultaneously expressing, or coordinately producing one or more RNAs or proteins transcribed from or encoded by beta-ketoacyl-ACP synthase I, beta-ketoacyl-ACP synthase IV, delta-9 desaturase, or CP4 EPSPS, plant cells and plants transformed with the same, and seeds, oil, and other products produced from the transformed plants.

Also provided by the present invention is a recombinant nucleic acid molecule comprising a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least one, preferably two, genes selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes; and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

Further provided by the present invention is a recombinant nucleic acid molecule comprising a first set of DNA sequences that is capable, when expressed in a host cell, of forming a dsRNA construct and suppressing the endogenous expression of at least one, preferably two, genes selected from the group consisting of *FAD2, FAD3*, and *FATB* genes, where the first set of DNA sequences comprises a first non-coding sequence that expresses a first RNA sequence that exhibits at least 90% identity to a non-coding region of a *FAD2* gene, a

first antisense sequence that expresses a first antisense RNA sequence capable of forming a double-stranded RNA molecule with the first RNA sequence, a second non-coding sequence that expresses a second RNA sequence that exhibits at least 90% identity to a non-coding region of a *FAD3* gene, and a second antisense sequence that expresses a second antisense RNA sequence capable of forming a double-stranded RNA molecule with the second RNA sequence; and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

The present invention provides methods of transforming plants with these recombinant nucleic acid molecules. The methods include a method of producing a transformed plant having seed with an increased oleic acid content, reduced saturated fatty acid content, and reduced polyunsaturated fatty acid content, comprising (A) transforming a plant cell with a recombinant nucleic acid molecule which comprises a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least one, preferably two, genes selected from the group consisting of *FAD2, FAD3,* and *FATB* genes, and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene; and (B) growing the transformed plant, where the transformed plant produces seed with an increased oleic acid content, reduced saturated fatty acid content, and reduced polyunsaturated fatty acid content relative to seed from a plant having a similar genetic background but lacking the recombinant nucleic acid molecule.

Further provided are methods of transforming plant cells with the recombinant nucleic acid molecules. The methods include a method of altering the oil composition of a plant cell comprising (A) transforming a plant cell with a recombinant nucleic acid molecule which comprises a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least one, preferably two, genes selected from the group consisting of *FAD2, FAD3,* and *FATB* genes, and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-

ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene; and (B) growing the plant cell under conditions where transcription of the first set of DNA sequences and the second set of DNA sequences is initiated, where the oil composition is altered relative to a plant cell with a similar genetic background but lacking the recombinant nucleic acid molecule.

The present invention also provides a transformed plant comprising a recombinant nucleic acid molecule which comprises a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least one, preferably two, genes selected from the group consisting of *FAD2, FAD3,* and *FATB* genes, and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene. Further provided by the present invention is a transformed soybean plant bearing seed, where the seed exhibits an oil composition which comprises 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids, and feedstock, plant parts, and seed derived from the plant.

The present invention provides a soybean seed exhibiting an oil composition comprising 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids, and also provides a soybean seed exhibiting an oil composition comprising 65 to 80% by weight oleic acid, 10 to 30% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight of saturated fatty acids. Also provided by the present invention are soyfoods comprising an oil composition which comprises 69 to 73% by weight oleic acid, 21 to 24% by weight linoleic acid, 0.5 to 3% by weight linolenic acid, and 2-3% by weight of saturated fatty acids.

The crude soybean oil provided by the present invention exhibits an oil composition comprising 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids. Another crude soybean oil provided by the present invention exhibits an oil composition comprising 65 to 80% by weight oleic acid, 10 to 30% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight of saturated fatty acids.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-4 each depict exemplary nucleic acid molecule configurations;

FIGS. 5 and 6 each depict illustrative configurations of a first set of DNA sequences; and

FIGS. 7-15 each depict nucleic acid molecules of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Description of the Nucleic Acid Sequences

SEQ ID NO: 1 is a nucleic acid sequence of a *FAD2-1A* intron 1.

SEQ ID NO: 2 is a nucleic acid sequence of a *FAD2-1B* intron 1.

SEQ ID NO: 3 is a nucleic acid sequence of a *FAD2-1B* promoter.

SEQ ID NO: 4 is a nucleic acid sequence of a *FAD2-1A* genomic clone.

SEQ ID NOs: 5 & 6 are nucleic acid sequences of a *FAD2-1A* 3' UTR and 5'UTR, respectively.

SEQ ID NOs: 7-13 are nucleic acid sequences of *FAD3-1A* introns 1, 2, 3A, 4, 5, 3B, and 3C, respectively.

SEQ ID NO: 14 is a nucleic acid sequence of a *FAD3-1C* intron 4.

SEQ ID NO: 15 is a nucleic acid sequence of a partial *FAD3-1A* genomic clone.

SEQ ID NOs: 16 & 17 are nucleic acid sequences of a *FAD3-1A* 3'UTR and 5'UTR, respectively.

SEQ ID NO: 18 is a nucleic acid sequence of a partial *FAD3-1B* genomic clone.

SEQ ID NOs: 19-25 are nucleic acid sequences of *FAD3-1B* introns 1, 2, 3A, 3B, 3C, 4, and 5, respectively.

SEQ ID NOs: 26 & 27 are nucleic acid sequences of a *FAD3-1B* 3'UTR and 5'UTR, respectively.

SEQ ID NO: 28 is a nucleic acid sequence of a *FATB* genomic clone.

SEQ ID NO: 29-35 are nucleic acid sequences of *FATB* introns I, II, III, IV, V, VI, and VII, respectively.

SEQ ID NOs: 36 & 37 are nucleic acid sequences of a *FATB* 3'UTR and 5'UTR, respectively.

SEQ ID NO: 38 is a nucleic acid sequence of a *Cuphea pulcherrima KAS I* gene.

SEQ ID NO: 39 is a nucleic acid sequence of a *Cuphea pulcherrima KAS IV* gene.

SEQ ID NOs: 40 & 41 are nucleic acid sequences of *Ricinus communis* and *Simmondsia chinensis* delta-9 desaturase genes, respectively.

Definitions

"ACP" refers to an acyl carrier protein moiety. "Altered seed oil composition" refers to a seed oil composition from a transgenic or transformed plant of the invention which has altered or modified levels of the fatty acids therein, relative to a seed oil from a plant having a similar genetic background but that has not been transformed. "Antisense suppression" refers to gene-specific silencing that is induced by the introduction of an antisense RNA molecule.

"Coexpression of more than one agent such as an mRNA or protein" refers to the simultaneous expression of an agent in overlapping time frames and in the same cell or tissue as another agent. "Coordinated expression of more than one agent" refers to the coexpression of more than one agent when the production of transcripts and proteins from such agents is carried out utilizing a shared or identical promoter. "Complement" of a nucleic acid sequence refers to the complement of the sequence along its complete length.

"Cosuppression" is the reduction in expression levels, usually at the level of RNA, of a particular endogenous gene or gene family by the expression of a homologous sense construct that is capable of transcribing mRNA of the same strandedness as the transcript of the endogenous gene. Napoli *et al., Plant Cell 2*:279-289 (1990); van der Krol *et al., Plant Cell 2*:291-299 (1990). "Crude soybean oil" refers to soybean oil that has been extracted from soybean seeds, but has not been refined, processed, or blended, although it may be degummed.

When referring to proteins and nucleic acids herein, "derived" refers to either directly (for example, by looking at the sequence of a known protein or nucleic acid and preparing a protein or nucleic acid having a sequence similar, at least in part, to the sequence of the known protein or nucleic acid) or indirectly (for example, by obtaining a protein or nucleic acid from an organism which is related to a known protein or nucleic acid) obtaining a protein or nucleic acid from a known protein or nucleic acid. Other methods of "deriving" a protein or nucleic acid from a known protein or nucleic acid are known to one of skill in the art.

"dsRNA", "dsRNAi" and "RNAi" all refer to gene-specific silencing that is induced by the introduction of a construct capable of forming a double-stranded RNA molecule. A "dsRNA molecule" and an "RNAi molecule" both refer to a double-stranded RNA molecule

capable, when introduced into a cell or organism, of at least partially reducing the level of an mRNA species present in a cell or a cell of an organism.

"Exon" refers to the normal sense of the term as meaning a segment of nucleic acid molecules, usually DNA, that encodes part of or all of an expressed protein.

"Fatty acid" refers to free fatty acids and fatty acyl groups.

"Gene" refers to a nucleic acid sequence that encompasses a 5' promoter region associated with the expression of the gene product, any intron and exon regions and 3' or 5' untranslated regions associated with the expression of the gene product. "Gene silencing" refers to the suppression of gene expression or down-regulation of gene expression.

A "gene family" is two or more genes in an organism which encode proteins that exhibit similar functional attributes, and a "gene family member" is any gene of the gene family found within the genetic material of the plant, *e.g.*, a "*FAD2* gene family member" is any *FAD2* gene found within the genetic material of the plant. An example of two members of a gene family are *FAD2-1* and *FAD2-2*. A gene family can be additionally classified by the similarity of the nucleic acid sequences. Preferably, a gene family member exhibits at least 60%, more preferably at least 70%, more preferably at least 80% nucleic acid sequence identity in the coding sequence portion of the gene.

"Heterologous" means not naturally occurring together. A "high oleic soybean seed" is a seed with oil having greater than 75% oleic acid present in the oil composition of the seed.

A nucleic acid molecule is said to be "introduced" if it is inserted into a cell or organism as a result of human manipulation, no matter how indirect. Examples of introduced nucleic acid molecules include, but are not limited to, nucleic acids that have been introduced into cells via transformation, transfection, injection, and projection, and those that have been introduced into an organism via methods including, but not limited to, conjugation, endocytosis, and phagocytosis.

"Intron" refers to the normal sense of the term as meaning a segment of nucleic acid molecules, usually DNA, that does not encode part of or all of an expressed protein, and which, in endogenous conditions, is transcribed into RNA molecules, but which is spliced out of the endogenous RNA before the RNA is translated into a protein. An "intron dsRNA molecule" and an "intron RNAi molecule" both refer to a double-stranded RNA molecule capable, when introduced into a cell or organism, of at least partially reducing the level of an mRNA species

present in a cell or a cell of an organism where the double-stranded RNA molecule exhibits sufficient identity to an intron of a gene present in the cell or organism to reduce the level of an mRNA containing that intron sequence.

A "low saturate" oil composition contains between 3.6 and 8 percent saturated fatty acids.

A "mid-oleic soybean seed" is a seed having between 50% and 85% oleic acid present in the oil composition of the seed.

The term "non-coding" refers to sequences of nucleic acid molecules that do not encode part or all of an expressed protein. Non-coding sequences include but are not limited to introns, promoter regions, 3' untranslated regions (3'UTRs), and 5' untranslated regions (5'UTRs).

A promoter that is "operably linked" to one or more nucleic acid sequences is capable of driving expression of one or more nucleic acid sequences, including multiple coding or non-coding nucleic acid sequences arranged in a polycistronic configuration.

"Physically linked" nucleic acid sequences are nucleic acid sequences that are found on a single nucleic acid molecule. A "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, and plant cells and progeny of the same. The term "plant cell" includes, without limitation, seed suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. "Plant promoters," include, without limitation, plant viral promoters, promoters derived from plants, and synthetic promoters capable of functioning in a plant cell to promote the expression of an mRNA.

A "polycistronic gene" or "polycistronic mRNA" is any gene or mRNA that contains transcribed nucleic acid sequences which correspond to nucleic acid sequences of more than one gene targeted for expression. It is understood that such polycistronic genes or mRNAs may contain sequences that correspond to introns, 5'UTRs, 3'UTRs, or combinations thereof, and that a recombinant polycistronic gene or mRNA might, for example without limitation, contain sequences that correspond to one or more UTRs from one gene and one or more introns from a second gene.

A "seed-specific promoter" refers to a promoter that is active preferentially or exclusively in a seed. "Preferential activity" refers to promoter activity that is substantially

greater in the seed than in other tissues, organs or organelles of the plant. "Seed-specific" includes without limitation activity in the aleurone layer, endosperm, and/or embryo of the seed.

"Sense intron suppression" refers to gene silencing that is induced by the introduction of a sense intron or fragment thereof. Sense intron suppression is described by Fillatti in PCT WO 01/14538 A2. "Simultaneous expression" of more than one agent such as an mRNA or protein refers to the expression of an agent at the same time as another agent. Such expression may only overlap in part and may also occur in different tissue or at different levels.

"Total oil level" refers to the total aggregate amount of fatty acid without regard to the type of fatty acid. "Transgene" refers to a nucleic acid sequence associated with the expression of a gene introduced into an organism. A transgene includes, but is not limited to, a gene endogenous or a gene not naturally occurring in the organism. A "transgenic plant" is any plant that stably incorporates a transgene in a manner that facilitates transmission of that transgene from a plant by any sexual or asexual method.

A "zero saturate" oil composition contains less than 3.6 percent saturated fatty acids.

When referring to proteins and nucleic acids herein, the use of plain capitals, *e.g.*, "FAD2", indicates a reference to an enzyme, protein, polypeptide, or peptide, and the use of italicized capitals, *e.g.*, "*FAD2*", is used to refer to nucleic acids, including without limitation genes, cDNAs, and mRNAs. A cell or organism can have a family of more than one gene encoding a particular enzyme, and the capital letter that follows the gene terminology (A, B, C) is used to designate the family member, *i.e.*, *FAD2-1A* is a different gene family member from *FAD2-1B*.

As used herein, any range set forth is inclusive of the end points of the range unless otherwise stated.

A. Agents

The agents of the invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid molecule to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic and thus involve the capacity of the agent to mediate a chemical reaction or response. The agents will preferably be "substantially purified." The term "substantially purified," as

used herein, refers to a molecule separated from substantially all other molecules normally associated with it in its native environmental conditions. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, greater than 75% free, preferably greater than 90% free, and most preferably greater than 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native environmental conditions.

The agents of the invention may also be recombinant. As used herein, the term "recombinant" means any agent (*e.g.*, including but limited to DNA, peptide), that is, or results, however indirectly, from human manipulation of a nucleic acid molecule. It is also understood that the agents of the invention may be labeled with reagents that facilitate detection of the agent, *e.g.*, fluorescent labels, chemical labels, and/or modified bases.

Agents of the invention include nucleic acid molecules that comprise a DNA sequence which is at least 50%, 60%, or 70% identical over their entire length to a plant coding region or non-coding region, or to a nucleic acid sequence that is complementary to a plant coding or non-coding region. More preferable are DNA sequences that are, over their entire length, at least 80% identical; at least 85% identical; at least 90% identical; at least 95% identical; at least 97% identical; at least 98% identical; at least 99% identical; or 100% identical to a plant coding region or non-coding region, or to a nucleic acid sequence that is complementary to a plant coding or non-coding region.

"Identity," as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more nucleic acid molecule sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or nucleic acid molecule sequences, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods including, but not limited to, those described in *Computational Molecular Biology*, Lesk, ed., Oxford University Press, New York 1988; *Biocomputing: Informatics and Genome Projects*, Smith, ed., Academic Press, New York 1993; *Computer Analysis of Sequence Data, Part I*, Griffin and Griffin, eds., Humana Press, New Jersey 1994; *Sequence Analysis in Molecular Biology*, von Heinje, Academic Press 1987; *Sequence Analysis Primer*, Gribskov and Devereux, eds.,

Stockton Press, New York 1991; and Carillo and Lipman, SIAM *J. Applied Math*, 48:1073 1988.

Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available programs. Computer programs which can be used to determine identity between two sequences include, but are not limited to, GCG; a suite of five BLAST programs, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN). The BLASTX program is publicly available from NCBI and other sources, *e.g.*, *BLAST Manual*, Altschul *et al.*, NCBI NLM NIH, Bethesda, MD 20894; Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990). The well-known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison typically include the following: Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48:443-453 (1970); Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992); Gap Penalty: 12; Gap Length Penalty: 4. A program that can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group ("GCG"), Madison, Wisconsin. The above parameters along with no penalty for end gap are the default parameters for peptide comparisons.

Parameters for nucleic acid molecule sequence comparison include the following: Algorithm: Needleman and Wunsch, *J. Mol. Bio.* 48:443-453 (1970); Comparison matrix: matches - +10; mismatches = 0; Gap Penalty: 50; Gap Length Penalty: 3. As used herein, "% identity" is determined using the above parameters as the default parameters for nucleic acid molecule sequence comparisons and the "gap" program from GCG, version 10.2.

Subsets of the nucleic acid sequences of the present invention include fragment nucleic acid molecules. "Fragment nucleic acid molecule" refers to a piece of a larger nucleic acid molecule, which may consist of significant portion(s) of, or indeed most of, the larger nucleic acid molecule, or which may comprise a smaller oligonucleotide having from about 15 to about 400 contiguous nucleotides and more preferably, about 15 to about 45 contiguous nucleotides, about 20 to about 45 contiguous nucleotides, about 15 to about 30 contiguous nucleotides, about 21 to about 30 contiguous nucleotides, about 21 to about 25 contiguous nucleotides, about 21 to about 24 contiguous nucleotides, about 19 to about 25 contiguous nucleotides, or

about 21 contiguous nucleotides. Fragment nucleic acid molecules may consist of significant portion(s) of, or indeed most of, a plant coding or non-coding region, or alternatively may comprise smaller oligonucleotides. In a preferred embodiment, a fragment shows 100% identity to the plant coding or non-coding region. In another preferred embodiment, a fragment comprises a portion of a larger nucleic acid sequence. In another aspect, a fragment nucleic acid molecule has a nucleic acid sequence that has at least 15, 25, 50, or 100 contiguous nucleotides of a nucleic acid molecule of the present invention. In a preferred embodiment, a nucleic acid molecule has a nucleic acid sequence that has at least 15, 25, 50, or 100 contiguous nucleotides of a plant coding or non-coding region.

In another aspect of the present invention, the DNA sequence of the nucleic acid molecules of the present invention can comprise sequences that differ from those encoding a polypeptide or fragment of the protein due to conservative amino acid changes in the polypeptide; the nucleic acid sequences coding for the polypeptide can therefore have sequence differences corresponding to the conservative changes. In a further aspect of the present invention, one or more of the nucleic acid molecules of the present invention differ in nucleic acid sequence from those for which a specific sequence is provided herein because one or more codons have been replaced with a codon that encodes a conservative substitution of the amino acid originally encoded.

Agents of the invention also include nucleic acid molecules that encode at least about a contiguous 10 amino acid region of a polypeptide of the present invention, more preferably at least about a contiguous 25, 40, 50, 100, or 125 amino acid region of a polypeptide of the present invention. Due to the degeneracy of the genetic code, different nucleotide codons may be used to code for a particular amino acid. A host cell often displays a preferred pattern of codon usage. Structural nucleic acid sequences are preferably constructed to utilize the codon usage pattern of the particular host cell. This generally enhances the expression of the structural nucleic acid sequence in a transformed host cell. Any of the above-described nucleic acid and amino acid sequences may be modified to reflect the preferred codon usage of a host cell or organism in which they are contained. Therefore, a contiguous 10 amino acid region of a polypeptide of the present invention could be encoded by numerous different nucleic acid sequences. Modification of a structural nucleic acid sequence for optimal codon usage in plants is described in U.S. Patent No. 5,689,052.

Agents of the invention include nucleic acid molecules. For example, without limitation, in an aspect of the present invention, the nucleic acid molecule of the present invention comprises an intron sequence of SEQ ID NO: 19, 20, 21, 22, 23, 25, 32, 33, 34, or 35 or fragments thereof or complements thereof. In another aspect of the invention, the nucleic acid molecule comprises a nucleic acid sequence, which when introduced into a cell or organism, is capable of suppressing the production of an RNA or protein while simultaneously expressing, coexpressing or coordinately expressing another RNA or protein. In an aspect of the invention, the nucleic acid molecule comprises a nucleic acid sequence, which when introduced into a cell or organism is capable of suppressing, at least partially reducing, reducing, substantially reducing, or effectively eliminating the expression of endogenous *FAD2*, *FAD3*, and/or *FATB* RNA while at the same time coexpressing, simultaneously expressing, or coordinately expressing a beta-ketoacyl-ACP synthase I, beta-ketoacyl-ACP synthase IV, delta-9 desaturase, and/or CP4 EPSPS RNA or protein.

By decreasing the amount of FAD2 and/or FAD3 available in a plant cell, a decreased percentage of polyunsaturated fatty acids such as linoleate (C18:2) and linolenate (C18:3) may be provided. Modifications in the pool of fatty acids available for incorporation into triacylglycerols may likewise affect the composition of oils in the plant cell. Thus, a decrease in expression of FAD2 and/or FAD3 may result in an increased proportion of mono-unsaturated fatty acids such as oleate (C18:1). When the amount of FATB is decreased in a plant cell, a decreased amount of saturated fatty acids such as palmitate and stearate may be provided. Thus, a decrease in expression of FATB may result in an increased proportion of unsaturated fatty acids such as oleate (18:1). The simultaneous suppression of FAD2, FAD3, and FATB expression thereby results in driving the FAS pathway toward an overall increase in mono-unsaturated fatty acids of 18-carbon length, such as oleate (C18:1). *See* U.S. Patent No. 5,955,650.

By increasing the amount of beta-ketoacyl-ACP synthase I (KAS I) and/or beta-ketoacyl-ACP synthase IV (KAS IV) available in a plant cell, a decreased percentage of 16:0-ACP may be provided, leading to an increased percentage of 18:0-ACP. A greater amount of 18:0-ACP in combination with the simultaneous suppression of one or more of FAD2, FAD3, and FATB, thereby helps drive the oil composition toward an overall increase in oleate (C18:1). By increasing the amount of delta-9 desaturase available in a plant cell, an increased

percentage of unsaturated fatty acids may be provided, resulting in an overall lowering of stearate and total saturates.

These combinations of increased and decreased enzyme expression may be manipulated to produce fatty acid compositions, including oils, having an increased oleate level, decreased linoleate, linolenate, stearate, and/or palmitate levels, and a decreased overall level of saturates. Enhancement of gene expression in plants may occur through the introduction of extra copies of coding sequences of the genes into the plant cell or, preferably, the incorporation of extra copies of coding sequences of the gene into the plant genome. Over-expression may also occur though increasing the activities of the regulatory mechanisms that regulate the expression of genes, *i.e.*, up-regulation of the gene expression.

Production of CP4 EPSPS in a plant cell provides the plant cell with resistance or tolerance to glyphosate, thereby providing a convenient method for identification of successful transformants via glyphosate-tolerant selection.

Suppression of gene expression in plants, also known as gene silencing, occurs at both the transcriptional level and post-transcriptional level. There are various methods for the suppression of expression of endogenous sequences in a host cell, including, but not limited to, antisense suppression, co-suppression, ribozymes, combinations of sense and antisense (double-stranded RNAi), promoter silencing, and DNA binding proteins such as zinc finger proteins. (*See, e.g.*, WO 98/53083 and WO 01/14538). Certain of these mechanisms are associated with nucleic acid homology at the DNA or RNA level. In plants, double-stranded RNA molecules can induce sequence-specific silencing. Gene silencing is often referred to as double stranded RNA ("dsRNAi") in plants, as RNA interference or RNAi in *Caenorhabditis elegans* and in animals, and as quelling in fungi.

In a preferred embodiment, the nucleic acid molecule of the present invention comprises (a) a first set of DNA sequences, each of which exhibits sufficient homology to one or more coding or non-coding sequences of a plant gene such that when it is expressed, it is capable of effectively eliminating, substantially reducing, or at least partially reducing the level of an mRNA transcript or protein encoded by the gene from which the coding or non-coding sequence was derived, or any gene which has homology to the target non-coding sequence, and (b) a second set of DNA sequences, each of which exhibits sufficient homology to a plant gene

so that when it is expressed, it is capable of at least partially enhancing, increasing, enhancing, or substantially enhancing the level of an mRNA transcript or protein encoded by the gene.

As used herein, "a reduction" of the level or amount of an agent such as a protein or mRNA means that the level or amount is reduced relative to a cell or organism lacking a DNA sequence capable of reducing the agent. For example, "at least a partial reduction" refers to a reduction of at least 25%, "a substantial reduction" refers to a reduction of at least 75%, and "an effective elimination" refers to a reduction of greater than 95%, all of which reductions in the level or amount of the agent are relative to a cell or organism lacking a DNA sequence capable of reducing the agent.

As used herein, "an enhanced" or "increased" level or amount of an agent such as a protein or mRNA means that the level or amount is higher than the level or amount of agent present in a cell, tissue or plant with a similar genetic background but lacking an introduced nucleic acid molecule encoding the protein or mRNA. For example, an "at least partially enhanced" level refers to an increase of at least 25%, and a "substantially enhanced" level refers to an increase of at least 100%, all of which increases in the level or amount of an agent are relative to the level or amount of agent that is present in a cell, tissue or plant with a similar genetic background but lacking an introduced nucleic acid molecule encoding the protein or mRNA.

When levels of an agent are compared, such a comparison is preferably carried out between organisms with a similar genetic background. Preferably, a similar genetic background is a background where the organisms being compared share 50% or greater, more preferably 75% or greater, and, even more preferably 90% or greater sequence identity of nuclear genetic material. In another preferred aspect, a similar genetic background is a background where the organisms being compared are plants, and the plants are isogenic except for any genetic material originally introduced using plant transformation techniques. Measurement of the level or amount of an agent may be carried out by any suitable method, non-limiting examples of which include comparison of mRNA transcript levels, protein or peptide levels, and/or phenotype, especially oil content. As used herein, mRNA transcripts include processed and non-processed mRNA transcripts, and proteins or peptides include proteins or peptides with or without any post-translational modification.

The DNA sequences of the first set of DNA sequences may be coding sequences, intron sequences, 3'UTR sequences, 5'UTR sequences, promoter sequences, other non-coding sequences, or any combination of the foregoing. The first set of DNA sequences encodes one or more sequences which, when expressed, are capable of selectively reducing either or both the protein and the transcript encoded by a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB*. In a preferred embodiment, the first set of DNA sequences is capable of expressing antisense RNA, in which the individual antisense sequences may be linked in one transcript, or may be in unlinked individual transcripts. In a further preferred embodiment, the first set of DNA sequences are physically linked sequences which are capable of expressing a single dsRNA molecule. In a different preferred embodiment, the first set of DNA sequences is capable of expressing sense cosuppresion RNA, in which the individual sense sequences may be linked in one transcript, or may be in unlinked individual transcripts. Exemplary embodiments of the first set of DNA sequences are described in Part B of the Detailed Description, and in the Examples.

The second set of DNA sequences encodes one or more sequences which, when expressed, are capable of increasing one or both of the protein and transcript encoded by a gene selected from the group consisting of beta-ketoacyl-ACP synthase I (*KAS I*), beta-ketoacyl-ACP synthase IV (*KAS IV*), delta-9 desaturase, and CP4 EPSPS. The DNA sequences of the second set of DNA sequences may be physically linked sequences. Exemplary embodiments of the second set of DNA sequences are described below in Parts C and D of the Detailed Description.

Thus, the present invention provides methods for altering the composition of fatty acids and compounds containing such fatty acids, such as oils, waxes, and fats. The present invention also provides methods for the production of particular fatty acids in host cell plants. Such methods employ the use of the expression cassettes described herein for the modification of the host plant cell's FAS pathway.

B. First Set of DNA Sequences

In an aspect of the present invention, a nucleic acid molecule comprises a first set of DNA sequences, which when introduced into a cell or organism, expresses one or more sequences capable of effectively eliminating, substantially reducing, or at least partially reducing the levels of mRNA transcripts or proteins encoded by one or more genes. Preferred

aspects include as a target an endogenous gene, a plant gene, and a non-viral gene. In an aspect of the present invention, a gene is a *FAD2*, *FAD3*, or *FATB* gene.

In an aspect, a nucleic acid molecule of the present invention comprises a DNA sequence which exhibits sufficient homology to one or more coding or non-coding sequences from a plant gene, which when introduced into a plant cell or plant and expressed, is capable of effectively eliminating, substantially reducing, or at least partially reducing the level of an mRNA transcript or protein encoded by the gene from which the coding or non-coding sequence(s) was derived. The DNA sequences of the first set of DNA sequences encode RNA sequences or RNA fragments which exhibit at least 90%, preferably at least 95%, more preferably at least 98%, most preferably at least 100% identity to a coding or non-coding region derived from the gene which is to be suppressed. Such percent identity may be to a nucleic acid fragment.

Preferably, the non-coding sequence is a 3' UTR, 5'UTR, or a plant intron from a plant gene. More preferably, the non-coding sequence is a promoter sequence, 3' UTR, 5'UTR, or a plant intron from a plant gene. The intron may be located between exons, or located within a 5' or 3' UTR of a plant gene.

The sequence(s) of the first set of DNA sequences may be designed to express a dsRNA construct, a sense suppression RNA construct, or an antisense RNA construct or any other suppression construct in order to achieve the desired effect when introduced into a plant cell or plant. Such DNA sequence(s) may be fragment nucleic acid molecules. In a preferred aspect, a dsRNA construct contains exon sequences, but the exon sequences do not correspond to a sufficient part of a plant exon to be capable of effectively eliminating, substantially reducing, or at least partially reducing the level of an mRNA transcript or protein encoded by the gene from which the exon was derived.

A plant intron can be any plant intron from a gene, whether endogenous or introduced. Nucleic acid sequences of such introns can be derived from a multitude of sources, including, without limitation, databases such as EMBL and Genbank which may be found on the Internet at ebi.ac.uk/swisprot/; expasy.ch/; embl-heidelberg.de/; and ncbi.nlm.nih.gov. Nucleic acid sequences of such introns can also be derived, without limitation, from sources such as the GENSCAN program which may be found on the Internet at genes.mit.edu/GENSCAN.html.

Additional introns may also be obtained by methods which include, without limitation, screening a genomic library with a probe of either known exon or intron sequences, comparing genomic sequence with its corresponding cDNA sequence, or cloning an intron such as a soybean intron by alignment to an intron from another organism, such as, for example, *Arabidopsis*. In addition, other nucleic acid sequences of introns will be apparent to one of ordinary skill in the art. The above-described methods may also be used to derive and obtain other non-coding sequences, including but not limited to, promoter sequences, 3'UTR sequences, and 5'UTR sequences.

A"*FAD2*", "Δ12 desaturase" or "omega-6 desaturase" gene encodes an enzyme (FAD2) capable of catalyzing the insertion of a double bond into a fatty acyl moiety at the twelfth position counted from the carboxyl terminus. The term "*FAD2-1*" is used to refer to a *FAD2* gene that is naturally expressed in a specific manner in seed tissue, and the term "*FAD2-2*" is used to refer a *FAD2* gene that is (a) a different gene from a *FAD2-1* gene and (b) is naturally expressed in multiple tissues, including the seed. Representative *FAD2* sequences include, without limitation, those set forth in U.S. Patent Application No. 10/176,149 filed on June 21, 2002, and in SEQ ID NOs: 1-6.

A "*FAD3*", "Δ15 desaturase" or "omega-3 desaturase" gene encodes an enzyme (FAD3) capable of catalyzing the insertion of a double bond into a fatty acyl moiety at the fifteenth position counted from the carboxyl terminus. The term "*FAD3-1*" is used to refer a *FAD3* gene family member that is naturally expressed in multiple tissues, including the seed. Representative *FAD3* sequences include, without limitation, those set forth in U.S. Patent Application No. 10/176,149 filed on June 21, 2002, and in SEQ ID NOs: 7-27.

A "*FATB*" or "palmitoyl-ACP thioesterase" gene encodes an enzyme (FATB) capable of catalyzing the hydrolytic cleavage of the carbon-sulfur thioester bond in the panthothene prosthetic group of palmitoyl-ACP as its preferred reaction. Hydrolysis of other fatty acid-ACP thioesters may also be catalyzed by this enzyme. Representative *FATB* sequences include, without limitation, those set forth in U.S. Provisional Application No. 60/390,185 filed on June 21, 2002; U.S. Patent Nos. 5,955,329; 5,723,761; 5,955,650; and 6,331,664; and SEQ ID NOs: 28-37.

C. Second Set of DNA Sequences

In an aspect of the present invention, a nucleic acid molecule comprises a second set of DNA sequences, which when introduced into a cell or organism, is capable of partially enhancing, increasing, enhancing, or substantially enhancing the levels of mRNA transcripts or proteins encoded by one or more genes. In an aspect of the present invention, a gene is an endogenous gene. In an aspect of the present invention, a gene is a plant gene. In another aspect of the present invention, a gene is a truncated gene where the truncated gene is capable of catalyzing the reaction catalyzed by the full gene. In an aspect of the present invention, a gene is a beta-ketoacyl-ACP synthase I, beta-ketoacyl-ACP synthase IV, delta-9 desaturase, or CP4 *EPSPS* gene.

A gene of the present invention can be any gene, whether endogenous or introduced. Nucleic acid sequences of such genes can be derived from a multitude of sources, including, without limitation, databases such as EMBL and Genbank which may be found on the Internet at ebi.ac.uk/swisprot/; expasy.ch/; embl-heidelberg.de/; and ncbi.nlm.nih.gov. Nucleic acid sequences of such genes can also be derived, without limitation, from sources such as the GENSCAN program which may be found on the Internet at genes.mit.edu/GENSCAN.html.

Additional genes may also be obtained by methods which include, without limitation, screening a genomic library or a cDNA library with a probe of either known gene sequences, cloning a gene by alignment to a gene or probe from another organism, such as, for example, *Arabidopsis*. In addition, other nucleic acid sequences of genes will be apparent to one of ordinary skill in the art. Additional genes may, for example without limitation, be amplified by polymerase chain reaction (PCR) and used in an embodiment of the present invention. In addition, other nucleic acid sequences of genes will be apparent to one of ordinary skill in the art.

Automated nucleic acid synthesizers may be employed for this purpose, and to make a nucleic acid molecule that has a sequence also found in a cell or organism. In lieu of such synthesis, nucleic acid molecules may be used to define a pair of primers that can be used with the PCR to amplify and obtain any desired nucleic acid molecule or fragment of a first gene.

A "*KAS I*" or "beta-ketoacyl-ACP synthase I" gene encodes an enzyme (KAS I) capable of catalyzing the elongation of a fatty acyl moiety up to palmitoyl-ACP (C16:0).

Representative *KAS I* sequences include, without limitation, those set forth in U.S. Patent No. 5,475,099 and PCT Publication WO 94/10189, and in SEQ ID NO: 38.

A "*KAS IV*" or "beta-ketoacyl-ACP synthase IV" gene encodes an enzyme (KAS IV) capable of catalyzing the condensation of medium chain acyl-ACPs and enhancing the production of 18:0-ACP. Representative *KAS IV* sequences include, without limitation, those set forth in PCT Publication WO 98/46776, and in SEQ ID NO: 39.

A "delta-9 desaturase" or "stearoyl-ACP desaturase" or "omega-9 desaturase" gene encodes an enzyme capable of catalyzing the insertion of a double bond into a fatty acyl moiety at the ninth position counted from the carboxyl terminus. A preferred delta-9 desaturase of the present invention is a plant or cyanobacterial delta-9 desaturase, and more preferably a delta-9 desaturase that is also found in an organism selected from the group consisting of *Cartharmus tinctorius, Ricinus communis, Simmonsia chinensis,* and *Brassica campestris*. Representative delta-9 desaturase sequences include, without limitation, those set forth in U.S. Patent No. 5,723,595, and SEQ ID NOs: 40-41.

A "CP4 *EPSPS*" or "CP4 5-enolpyruvylshikimate-3-phosphate synthase" gene encodes an enzyme (CP4 EPSPS) capable of conferring a substantial degree of glyphosate resistance upon the plant cell and plants generated therefrom. The CP4 *EPSPS* sequence may be a CP4 *EPSPS* sequence derived from *Agrobacterium tumefaciens sp.* CP4 or a variant or synthetic form thereof, as described in U.S. Patent No. 5,633,435. Representative CP4 *EPSPS* sequences include, without limitation, those set forth in U.S. Patent Nos. 5,627,061 and 5,633,435.

D. Recombinant Vectors and Constructs

One or more of the nucleic acid constructs of the invention may be used in plant transformation or transfection. The levels of products such as transcripts or proteins may be increased or decreased throughout an organism such as a plant or localized in one or more specific organs or tissues of the organism. For example the levels of products may be increased or decreased in one or more of the tissues and organs of a plant including without limitation: roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. A preferred organ is a seed. For example, exogenous genetic material may be transferred into a plant cell and the plant cell regenerated into a whole, fertile or sterile plant or plant part.

"Exogenous genetic material" is any genetic material, whether naturally occurring or otherwise, from any source that is capable of being inserted into any organism. Such

exogenous genetic material includes, without limitation, nucleic acid molecules and constructs of the present invention. Exogenous genetic material may be transferred into a host cell by the use of a DNA vector or construct designed for such a purpose. Design of such a vector is generally within the skill of the art (*See, e.g., Plant Molecular Biology: A Laboratory Manual*, Clark (ed.), Springer, New York (1997)).

A construct or vector may include a promoter functional in a plant cell, or a plant promoter, to express a nucleic acid molecule of choice. A number of promoters that are active in plant cells have been described in the literature, and the CaMV 35S and FMV promoters are preferred for use in plants. Preferred promoters are enhanced or duplicated versions of the CaMV 35S and FMV 35S promoters. Odell *et al.*, *Nature* 313: 810-812 (1985); U.S. Patent No. 5,378,619. Additional promoters that may be utilized are described, for example, in U.S. Patents 5,378,619; 5,391,725; 5,428,147; 5,447,858; 5,608,144; 5,608,144; 5,614,399; 5,633,441; 5,633,435; and 4,633,436. In addition, a tissue specific enhancer may be used.

Particularly preferred promoters can also be used to express a nucleic acid molecule of the present invention in seeds or fruits. Indeed, in a preferred embodiment, the promoter used is a seed specific promoter. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al., Seed Sci. Res.* 1:209-219 (1991)), phaseolin, stearoyl-ACP desaturase, 7Sα, 7sα' (Chen *et al., Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986)), USP, arcelin and oleosin. Preferred promoters for expression in the seed are 7Sα, 7sα', napin, and *FAD2-1A* promoters.

Constructs or vectors may also include other genetic elements, including but not limited to, 3' transcriptional terminators, 3' polyadenylation signals, other untranslated nucleic acid sequences, transit or targeting sequences, selectable or screenable markers, promoters, enhancers, and operators. Constructs or vectors may also contain a promoterless gene that may utilize an endogenous promoter upon insertion.

Nucleic acid molecules that may be used in plant transformation or transfection may be any of the nucleic acid molecules of the present invention. It is not intended that the present invention be limited to the illustrated embodiments. Exemplary nucleic acid molecules have been described in Part A of the Detailed Description, and further non-limiting exemplary nucleic acid molecules are described below and illustrated in FIGS. 1-4, and in the Examples.

Referring now to the drawings, embodiments of the nucleic acid molecule of the present invention are shown in FIGS. 1-4. As described above, the nucleic acid molecule comprises (a) a first set of DNA sequences and (b) a second set of DNA sequences, which are located on one or more T-DNA regions, each of which is flanked by a right border and a left border. Within the T-DNA regions the direction of transcription is shown by arrows. The nucleic acid molecules described may have their DNA sequences arranged in monocistronic or polycistronic configurations. Preferred configurations include a configuration in which the first set of DNA sequences and the second set of DNA sequences are located on a single T-DNA region.

In each of the illustrated embodiments, the first set of DNA sequences comprises one or more sequences which when expressed are capable of selectively reducing one or both of the protein and transcript encoded by a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB*. Preferably each sequence in the first set of DNA sequences is capable, when expressed, of suppressing the expression of a different gene, including without limitation different gene family members. The sequences may include coding sequences, intron sequences, 3'UTR sequences, 5'UTR sequences, other non-coding sequences, or any combination of the foregoing. The first set of DNA sequences may be expressed in any suitable form, including as a dsRNA construct, a sense cosuppression construct, or as an antisense construct. The first set of DNA sequences is operably linked to at least one promoter which drives expression of the sequences, which can be any promoter functional in a plant, or any plant promoter. Preferred promoters include, but are not limited to, a napin promoter, a 7Sα promoter, a 7sα' promoter, an arcelin promoter, or a *FAD2-1A* promoter.

The second set of DNA sequences comprises coding sequences, each of which is a DNA sequence that encodes a sequence that when expressed is capable of increasing one or both of the protein and transcript encoded by a gene selected from the group consisting of *KAS I*, *KAS IV*, delta-9 desaturase, and CP4 *EPSPS*. Each coding sequence is associated with a promoter, which can be any promoter functional in a plant, or any plant promoter. Preferred promoters for use in the second set of DNA sequences are an FMV promoter and/or seed-specific promoters. Particularly preferred seed-specific promoters include, but are not limited to, a napin promoter, a 7Sα promoter, a 7sα' promoter, an arcelin promoter, a delta-9 desaturase promoter, or a *FAD2-1A* promoter.

In the embodiments depicted in FIGS. 1 and 2, the first set of DNA sequences, when expressed, is capable of forming a dsRNA molecule that is capable of suppressing the expression of one or both of the protein and transcript encoded by, or transcribed from, a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB*. The first set of DNA sequences depicted in FIG. 1 comprises three non-coding sequences, each of which expresses an RNA sequence (not shown) that exhibits identity to a non-coding region of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The non-coding sequences each express an RNA sequence that exhibits at least 90% identity to a non-coding region of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The first set of DNA sequences also comprises three antisense sequences, each of which expresses an antisense RNA sequence (not shown) that is capable of forming a double-stranded RNA molecule with its respective corresponding RNA sequence (as expressed by the non-coding sequences).

The non-coding sequences may be separated from the antisense sequences by a spacer sequence, preferably one that promotes the formation of a dsRNA molecule. Examples of such spacer sequences include those set forth in Wesley *et al.*, *supra*, and Hamilton *et al.*, *Plant J.*, *15*:737-746 (1988). In a preferred aspect, the spacer sequence is capable of forming a hairpin structure as illustrated in Wesley *et al.*, *supra*. Particularly preferred spacer sequences in this context are plant introns or parts thereof. A particularly preferred plant intron is a spliceable intron. Spliceable introns include, but are not limited to, an intron selected from the group consisting of PDK intron, *FAD3-1A or FAD3-1B* intron #5, *FAD3* intron #1, *FAD3* intron #3A, *FAD3* intron #3B, *FAD3* intron #3C, *FAD3* intron #4, *FAD3* intron #5, *FAD2* intron #1, and *FAD2-2* intron. Preferred spliceable introns include, but are not limited to, an intron selected from the group consisting of *FAD3* intron #1, *FAD3* intron #3A, *FAD3* intron #3B, *FAD3* intron #3C, and *FAD3* intron #5. Other preferred spliceable introns include, but are not limited to, a spliceable intron that is about 0.75 kb to about 1.1 kb in length and is capable of facilitating an RNA hairpin structure. One non-limiting example of a particularly preferred spliceable intron is *FAD3* intron #5.

Referring now to FIG. 1, the nucleic acid molecule comprises two T-DNA regions, each of which is flanked by a right border and a left border. The first T-DNA region comprises the first set of DNA sequences that is operably linked to a promoter, and the first T-DNA region further comprises a first part of the second set of DNA sequences that comprises a first

promoter operably linked to a first coding sequence, and a second promoter operably linked to a second coding sequence. The second T-DNA region comprises a second part of the second set of DNA sequences that comprises a third promoter operably linked to a third coding sequence. In a preferred embodiment depicted in FIG. 2, the nucleic acid molecule comprises a single T-DNA region, which is flanked by a right border and a left border. The first and second sets of DNA sequences are all located on the single T-DNA region.

In the dsRNA-expressing embodiments shown in FIGS. 1 and 2, the order of the sequences may be altered from that illustrated and described, however the non-coding sequences and the antisense sequences preferably are arranged around the spacer sequence such that, when expressed, the first non-coding sequence can hybridize to the first antisense sequence, the second non-coding sequence can hybridize to the second antisense sequence, and the third non-coding sequence can hybridize to the third antisense sequence such that a single dsRNA molecule can be formed. Preferably the non-coding sequences are in a sense orientation, and the antisense sequences are in an antisense orientation relative to the promoter. The numbers of non-coding, antisense, and coding sequences, and the various relative positions thereof on the T-DNA region(s) may also be altered in any manner suitable for achieving the goals of the present invention.

Referring now to FIGS. 3 and 4, the illustrated nucleic acid molecule comprises a T-DNA region flanked by a right border and a left border, on which are located the first and second sets of DNA sequences. The first set of DNA sequences is operably linked to a promoter and a transcriptional termination signal. The second set of DNA sequences that comprises a first promoter operably linked to a first coding sequence, a second promoter operably linked to a second coding sequence, and a third promoter operably linked to a third coding sequence. The transcriptional termination signal can be any transcriptional termination signal functional in a plant, or any plant transcriptional termination signal. Preferred transcriptional termination signals include, but are not limited to, a pea Rubisco E9 3' sequence, a *Brassica* napin 3' sequence, a *tml* 3' sequence, and a *nos* 3' sequence.

In the embodiment depicted in FIG. 3, the first set of DNA sequences, when expressed, is capable of forming a sense cosuppression construct that is capable of suppressing the expression of one or more proteins or transcripts encoded by, or derived from, a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB*. The first set of DNA sequences

comprises three non-coding sequences, each of which expresses an RNA sequence (not shown) that exhibits identity to one or more non-coding region(s) of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The non-coding sequences each express an RNA sequence that exhibits at least 90% identity to one or more non-coding region(s) of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The order of the non-coding sequences within the first set of DNA sequences may be altered from that illustrated and described herein, but the non-coding sequences are arranged in a sense orientation relative to the promoter.

FIG. 4 depicts an embodiment in which the first set of DNA sequences, when expressed, is capable of forming an antisense construct that is capable of suppressing the expression of one or more proteins or transcripts encoded by, or derived from, a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB*. The first set of DNA sequences comprises three antisense sequences, each of which expresses an antisense RNA sequence (not shown) that exhibits identity to one or more non-coding region(s) of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The antisense sequences each express an antisense RNA sequence that exhibits at least 90% identity to one or more non-coding region(s) of a gene selected from the group consisting of *FAD2*, *FAD3*, and *FATB* genes. The order of the antisense sequences within the first set of DNA sequences may be altered from that illustrated and described herein, but the antisense sequences are arranged in an antisense orientation relative to the promoter.

The above-described nucleic acid molecules are preferred embodiments which achieve the objects, features and advantages of the present invention. It is not intended that the present invention be limited to the illustrated embodiments. The arrangement of the sequences in the first and second sets of DNA sequences within the nucleic acid molecule is not limited to the illustrated and described arrangements, and may be altered in any manner suitable for achieving the objects, features and advantages of the present invention as described herein and illustrated in the accompanying drawings.

E. Transgenic Organisms, and Methods for Producing Same

Any of the nucleic acid molecules and constructs of the invention may be introduced into a plant or plant cell in a permanent or transient manner. Preferred nucleic acid molecules and constructs of the present invention are described above in Parts A through D of the

Detailed Description, and in the Examples. Another embodiment of the invention is directed to a method of producing transgenic plants which generally comprises the steps of selecting a suitable plant or plant cell, transforming the plant or plant cell with a recombinant vector, and obtaining a transformed host cell.

In a preferred embodiment the plant or cell is, or is derived from, a plant involved in the production of vegetable oils for edible and industrial uses. Especially preferred are temperate oilseed crops. Plants of interest include, but are not limited to, rapeseed (canola and High Erucic Acid varieties), maize, soybean, crambe, mustard, castor bean, peanut, sesame, cotton, linseed, safflower, oil palm, flax, sunflower, and coconut. The invention is applicable to monocotyledonous or dicotyledonous species alike, and will be readily applicable to new and/or improved transformation and regulatory techniques.

Methods and technology for introduction of DNA into plant cells are well known to those of skill in the art, and virtually any method by which nucleic acid molecules may be introduced into a cell is suitable for use in the present invention. Non-limiting examples of suitable methods include: chemical methods; physical methods such as microinjection, electroporation, the gene gun, microprojectile bombardment, and vacuum infiltration; viral vectors; and receptor-mediated mechanisms. Other methods of cell transformation can also be used and include but are not limited to introduction of DNA into plants by direct DNA transfer into pollen, by direct injection of DNA into reproductive organs of a plant, or by direct injection of DNA into the cells of immature embryos followed by the rehydration of desiccated embryos.

Agrobacterium-mediated transfer is a widely applicable system for introducing genes into plant cells. *See, e.g.*, Fraley *et al., Bio/Technology 3*:629-635 (1985); Rogers *et al., Methods Enzymol. 153*:253-277 (1987). The region of DNA to be transferred is defined by the border sequences and intervening DNA is usually inserted into the plant genome. Spielmann *et al., Mol. Gen. Genet. 205*:34 (1986). Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations. Klee *et al.*, In: *Plant DNA Infectious Agents*, Hohn and Schell (eds.), Springer-Verlag, New York, pp. 179-203 (1985).

The regeneration, development and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art. *See generally,*

Maliga *et al., Methods in Plant Molecular Biology,* Cold Spring Harbor Press (1995); Weissbach and Weissbach, In: *Methods for Plant Molecular Biology*, Academic Press, San Diego, CA (1988). Plants of the present invention can be part of or generated from a breeding program, and may also be reproduced using apomixis. Methods for the production of apomictic plants are known in the art. *See, e.g.*, U.S. Patent 5,811,636.

In a preferred embodiment, a plant of the present invention that includes nucleic acid sequences which when expressed are capable of selectively reducing the level of a FAD2, FAD3, and/or FATB protein, and/or a *FAD2*, *FAD3*, and/or *FATB* transcript is mated with another plant of the present invention that includes nucleic acid sequences which when expressed are capable of overexpressing another enzyme. Preferably the other enzyme is selected from the group consisting of beta-ketoacyl-ACP synthase I, beta-ketoacyl-ACP synthase IV, delta-9 desaturase, and CP4 *EPSPS*.

F. Products of the Present Invention

The plants of the present invention may be used in whole or in part. Preferred plant parts include reproductive or storage parts. The term "plant parts" as used herein includes, without limitation, seed, endosperm, ovule, pollen, roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers. In a particularly preferred embodiment of the present invention, the plant part is a seed.

Any of the plants or parts thereof of the present invention may be processed to produce a feed, meal, protein, or oil preparation. A particularly preferred plant part for this purpose is a seed. In a preferred embodiment the feed, meal, protein or oil preparation is designed for livestock animals, fish or humans, or any combination. Methods to produce feed, meal, protein and oil preparations are known in the art. *See, e.g.*, U.S. Patents 4,957,748, 5,100,679, 5,219,596, 5,936,069, 6,005,076, 6,146,669, and 6,156,227. In a preferred embodiment, the protein preparation is a high protein preparation. Such a high protein preparation preferably has a protein content of greater than 5% w/v, more preferably 10% w/v, and even more preferably 15% w/v.

In a preferred oil preparation, the oil preparation is a high oil preparation with an oil content derived from a plant or part thereof of the present invention of greater than 5% w/v, more preferably 10% w/v, and even more preferably 15% w/v. In a preferred embodiment the oil preparation is a liquid and of a volume greater than 1, 5, 10 or 50 liters. The present

invention provides for oil produced from plants of the present invention or generated by a method of the present invention. Such an oil may exhibit enhanced oxidative stability. Also, such oil may be a minor or major component of any resultant product.

Moreover, such oil may be blended with other oils. In a preferred embodiment, the oil produced from plants of the present invention or generated by a method of the present invention constitutes greater than 0.5%, 1%, 5%, 10%, 25%, 50%, 75% or 90% by volume or weight of the oil component of any product. In another embodiment, the oil preparation may be blended and can constitute greater than 10%, 25%, 35%, 50% or 75% of the blend by volume. Oil produced from a plant of the present invention can be admixed with one or more organic solvents or petroleum distillates.

Seeds of the plants may be placed in a container. As used herein, a container is any object capable of holding such seeds. A container preferably contains greater than about 500, 1,000, 5,000, or 25,000 seeds where at least about 10%, 25%, 50%, 75% or 100% of the seeds are derived from a plant of the present invention. The present invention also provides a container of over about 10,000, more preferably about 20,000, and even more preferably about 40,000 seeds where over about 10%, more preferably about 25%, more preferably 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention. The present invention also provides a container of over about 10 kg, more preferably about 25 kg, and even more preferably about 50 kg seeds where over about 10%, more preferably about 25%, more preferably about 50% and even more preferably about 75% or 90% of the seeds are seeds derived from a plant of the present invention.

G. Oil Compositions

For many oil applications, saturated fatty acid levels are preferably less than 8% by weight, and more preferably about 2-3% by weight. Saturated fatty acids have high melting points which are undesirable in many applications. When used as a feedstock for fuel, saturated fatty acids cause clouding at low temperatures, and confer poor cold flow properties such as pour points and cold filter plugging points to the fuel. Oil products containing low saturated fatty acid levels may be preferred by consumers and the food industry because they are perceived as healthier and/or may be labeled as "saturated fat free" in accordance with FDA guidelines. In addition, low saturate oils reduce or eliminate the need to winterize the oil for food applications such as salad oils. In biodiesel and lubricant applications oils with low

saturated fatty acid levels confer improved cold flow properties and do not cloud at low temperatures.

The factors governing the physical properties of a particular oil are complex. Palmitic, stearic and other saturated fatty acids are typically solid at room temperature, in contrast to the unsaturated fatty acids, which remain liquid. Because saturated fatty acids have no double bonds in the acyl chain, they remain stable to oxidation at elevated temperatures. Saturated fatty acids are important components in margarines and chocolate formulations, but for many food applications, reduced levels of saturated fatty acids are desired.

Oleic acid has one double bond, but is still relatively stable at high temperatures, and oils with high levels of oleic acid are suitable for cooking and other processes where heating is required. Recently, increased consumption of high oleic oils has been recommended, because oleic acid appears to lower blood levels of low density lipoproteins ("LDLs") without affecting levels of high density lipoproteins ("HDLs"). However, some limitation of oleic acid levels is desirable, because when oleic acid is degraded at high temperatures, it creates negative flavor compounds and diminishes the positive flavors created by the oxidation of linoleic acid. Neff *et al.*, *JAOCS*, 77 :1303-1313 (2000); Warner *et al.*, *J. Agric. Food Chem.* 49:899-905 (2001). Preferred oils have oleic acid levels that are 65-85% or less by weight, in order to limit off-flavors in food applications such as frying oil and fried food. Other preferred oils have oleic acid levels that are greater than 55% by weight in order to improve oxidative stability.

Linoleic acid is a major polyunsaturated fatty acid in foods and is an essential nutrient for humans. It is a desirable component for many food applications because it is a major precursor of fried food flavor substances such as 2,4 decadienal, which make fried foods taste good. However, linoleic acid has limited stability when heated. Preferred food oils have linoleic acid levels that are 10% or greater by weight, to enhance the formation of desirable fried food flavor substances, and also are 25% or less by weight, so that the formation of off-flavors is reduced. Linoleic acid also has cholesterol-lowering properties, although dietary excess can reduce the ability of human cells to protect themselves from oxidative damage, thereby increasing the risk of cardiovascular disease. Toborek *et al.*, *Am J. Clin. J.* 75:119-125 (2002). *See generally Flavor Chemistry of Lipid Foods*, editors D.B. Min & T.H. Smouse, Am Oil Chem. Soc., Champaign, IL (1989).

Linoleic acid, having a lower melting point than oleic acid, further contributes to improved cold flow properties desirable in biodiesel and biolubricant applications. Preferred oils for most applications have linoleic acid levels of 30% or less by weight, because the oxidation of linoleic acid limits the useful storage or use-time of frying oil, food, feed, fuel and lubricant products. *See generally, Physical Properties of Fats, Oils, and Emulsifiers*, ed. N. Widlak, AOCS Press (1999); Erhan & Asadauskas, *Lubricant Basestocks from Vegetable Oils, Industrial Crops and Products,* 11:277-282 (2000). In addition, high linoleic acid levels in cattle feed can lead to undesirably high levels of linoleic acid in the milk of dairy cattle, and therefore poor oxidative stability and flavor. Timmons *et al., J. Dairy Sci.* 84:2440-2449 (2001). A broadly useful oil composition has linoleic acid levels of 10-25% by weight.

Linolenic acid is also an important component of the human diet. It is used to synthesize the ω-3 family of long-chain fatty acids and the prostaglandins derived therefrom. However, its double bonds are highly susceptible to oxidation, so that oils with high levels of linolenic acid deteriorate rapidly on exposure to air, especially at high temperatures. Partial hydrogenation of such oils is often necessary before they can be used in food products to retard the formation of off-flavors and rancidity when the oil is heated, but hydrogenation creates unhealthy *trans* fatty acids which can contribute to cardiovascular disease. To achieve improved oxidative stability, and reduce the need to hydrogenate oil, preferred oils have linolenic acid levels that are 8% or less by weight, 6% or less, 4% or less, and more preferably 0.5-2% by weight of the total fatty acids in the oil of the present invention.

The oil of the present invention can be a blended oil, synthesized oil or in a preferred embodiment an oil generated from an oilseed having an appropriate oil composition. In a preferred embodiment, the oil is a soybean oil. The oil can be a crude oil such as crude soybean oil, or can be a processed oil, for example the oil can be refined, bleached, deodorized, and/or winterized. As used herein, "refining" refers to a process of treating natural or processed fat or oil to remove impurities, and may be accomplished by treating fat or oil with caustic soda, followed by centrifugation, washing with water, and heating under vacuum. "Bleaching" refers to a process of treating a fat or oil to remove or reduce the levels of coloring materials in the fat or oil. Bleaching may be accomplished by treating fat or oil with activated charcoal or Fullers (diatomaceous) earth. "Deodorizing" refers to a process of removing components from a fat or oil that contribute objectionable flavors or odors to the end product,

and may be accomplished by use of high vacuum and superheated steam washing. "Winterizing" refers to a process of removing saturated glycerides from an oil, and may be accomplished by chilling and removal of solidified portions of fat from an oil.

A preferred oil of the present invention has a low saturate oil composition, or a zero saturate oil composition. In other preferred embodiments, oils of the present invention have increased oleic acid levels, reduced saturated fatty levels, and reduced polyunsaturated fatty acid levels. In a preferred embodiment, the oil is a soybean oil. The percentages of fatty acid content, or fatty acid levels, used herein refer to percentages by weight.

In a first embodiment, an oil of the present invention preferably has an oil composition that is 55 to 80% oleic acid, 10 to 40% linoleic acid, 6% or less linolenic acid, and 2 to 8% saturated fatty acids; more preferably has an oil composition that is 55 to 80% oleic acid, 10 to 39% linoleic acid, 4.5% or less linolenic acid, and 3 to 6% saturated fatty acids; and even more preferably has an oil composition that is 55 to 80% oleic acid, 10 to 39% linoleic acid, 3.0% or less linolenic acid, and 2 to 3.6% saturated fatty acids.

In a second embodiment, an oil of the present invention preferably has an oil composition that is 65 to 80% oleic acid, 10 to 30% linoleic acid, 6% or less linolenic acid, and 2 to 8% saturated fatty acids; more preferably has an oil composition that is 65 to 80% oleic acid, 10 to 29% linoleic acid, 4.5% or less linolenic acid, and 3 to 6% saturated fatty acids; and even more preferably has an oil composition that is 65 to 80% oleic acid, 10 to 29% linoleic acid, 3.0% or less linolenic acid, and 2 to 3.6% saturated fatty acids.

In other embodiments, the percentage of oleic acid is 50% or greater; 55% or greater; 60% or greater; 65% or greater; 70% or greater; 75% or greater; or 80% or greater; or is a range from 50 to 80%; 55 to 80%; 55 to 75%; 55 to 65%; 65 to 80%; 65 to 75%; 65 to 70%; or 69 to 73%. Suitable percentage ranges for oleic acid content in oils of the present invention also include ranges in which the lower limit is selected from the following percentages: 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 percent; and the upper limit is selected from the following percentages: 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 percent.

In these other embodiments, the percentage of linoleic acid in an oil of the present invention is a range from 10 to 40%; 10 to 39%; 10 to 30%; 10 to 29%; 10 to 28%; 10 to 25%;

10 to 21%; 10 to 20%; 11 to 30%; 12 to 30%; 15 to 25%; 20 to 25%; 20 to 30%; or 21 to 24%. Suitable percentage ranges for linoleic acid content in oils of the present invention also include ranges in which the lower limit is selected from the following percentages: 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 percent; and the upper limit is selected from the following percentages: 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 percent.

In these other embodiments, the percentage of linolenic acid in an oil of the present invention is 10% or less; 9% or less; 8% or less; 7% or less; 6% or less; 5% or less; 4.5% or less; 4% or less; 3.5% or less; 3% or less; 3.0% or less; 2.5% or less; or 2% or less; or is a range from 0.5 to 2%; 0.5 to 3%; 0.5 to 4.5%; 0.5% to 6%; 3 to 5%; 3 to 6%; 3 to 8%; 1 to 2%; 1 to 3%; or 1 to 4%. In these other embodiments, the percentage of saturated fatty acids in an oil composition of the present invention is 15% or less; 14% or less; 13% or less; 12% or less, 11% or less; 10% or less; 9% or less; 8% or less; 7% or less; 6% or less; 5% or less; 4% or less; or 3.6% or less; or is a range from 2 to 3%; 2 to 3.6%; 2 to 4%; 2 to 8%; 3 to 15%; 3 to 10%; 3 to 8%; 3 to 6%; 3.6 to 7%; 5 to 8%; 7 to 10%; or 10 to 15%.

An oil of the present invention is particularly suited to use as a cooking or frying oil. Because of its reduced polyunsaturated fatty acid content, the oil of the present invention does not require the extensive processing of typical oils because fewer objectionable odorous and colorant compounds are present. In addition, the low saturated fatty acid content of the present oil improves the cold flow properties of the oil, and obviates the need to heat stored oil to prevent it from crystallizing or solidifying. Improved cold flow also enhances drainage of oil from fried food material once it has been removed from frying oil, thereby resulting in a lower fat product. *See* Bouchon *et al., J. Food Science 66*: 918-923 (2001). The low levels of linolenic acid in the present oil are particularly advantageous in frying to reduce off-flavors.

The present oil is also well-suited for use as a salad oil (an oil that maintains clarity at refrigerator temperatures of 40-50 degrees Fahrenheit). Its improved clarity at refrigerator temperatures, due to its low saturated fatty acid and moderate linoleic acid content, reduces or eliminates the need to winterize the oil before use as a salad oil.

In addition, the moderate linoleic and low linolenic acid content of the present oil make it well-suited for the production of shortening, margarine and other semi-solid vegetable fats used in foodstuffs. Production of these fats typically involves hydrogenation of unsaturated

oils such as soybean oil, corn oil, or canola oil. The increased oxidative and flavor stability of the present oil mean that it need not be hydrogenated to the extent that typical vegetable oil is for uses such as margarine and shortening, thereby reducing processing costs and the production of unhealthy *trans* isomers.

An oil of the present invention is also suitable for use as a feedstock to produce biodiesel, particularly because biodiesel made from such an oil has improved cold flow, improved ignition quality (cetane number), improved oxidative stability, and reduced nitric oxide emissions. Biodiesel is an alternative diesel fuel typically comprised of methyl esters of saturated, monounsaturated, and polyunsaturated C_{16}-C_{22} fatty acids. Cetane number is a measure of ignition quality – the shorter the ignition delay time of fuel in the engine, the higher the cetane number. The ASTM standard specification for biodiesel fuel (D 6751-02) requires a minimum cetane number of 47.

The use of biodiesel in conventional diesel engines results in substantial reductions of pollutants such as sulfates, carbon monoxide, and particulates compared to petroleum diesel fuel, and use in school buses can greatly reduce children's exposure to toxic diesel exhaust. A limitation to the use of 100% conventional biodiesel as fuel is the high cloud point of conventional soy biodiesel (2 degrees C) compared to number 2 diesel (-16 degrees C). Dunn *et al., Recent. Res. Devel. in Oil Chem.*, 1:31-56 (1997). Biodiesel made from oil of the present invention has an improved (reduced) cloud point and cold filter plugging point, and may also be used in blends to improve the cold-temperature properties of biodiesel made from inexpensive but highly saturated sources of fat such as animal fats (tallow, lard, chicken fat) and palm oil. Biodiesel can also be blended with petroleum diesel at any level.

Biodiesel is typically obtained by extracting, filtering and refining soybean oil to remove free fats and phospholipids, and then transesterifying the oil with methanol to form methyl esters of the fatty acids. *See, e.g.*, U.S. Patent No. 5,891,203. The resultant soy methyl esters are commonly referred to as "biodiesel." The oil of the present invention may also be used as a diesel fuel without the formation of methyl esters, such as, for example, by mixing acetals with the oil. *See, e.g.*, U.S. Patent No. 6,013,114. Due to its improved cold flow and oxidative stability properties, the oil of the present invention is also useful as a lubricant, and as a diesel fuel additive. *See, e.g.*, U.S. Patent Nos. 5,888,947, 5,454,842 and 4,557,734.

Soybeans, and oils of the present invention are also suitable for use in a variety of soyfoods made from whole soybeans, such as soymilk, soy nut butter, natto, and tempeh, and soyfoods made from processed soybeans and soybean oil, including soybean meal, soy flour, soy protein concentrate, soy protein isolates, texturized soy protein concentrate, hydrolyzed soy protein, whipped topping, cooking oil, salad oil, shortening, and lecithin. Whole soybeans are also edible, and are typically sold to consumers raw, roasted, or as edamamé. Soymilk, which is typically produced by soaking and grinding whole soybeans, may be consumed as is, spray-dried, or processed to form soy yogurt, soy cheese, tofu, or yuba. The present soybean or oil may be advantageously used in these and other soyfoods because of its improved oxidative stability, the reduction of off-flavor precursors, and its low saturated fatty acid level.

The following examples are illustrative and not intended to be limiting in any way.

EXAMPLES

Example 1 Suppression Constructs

1A. FAD2-1 Constructs

The *FAD2-1A* intron (SEQ ID NO: 1) is cloned into the expression cassette, pCGN3892, in sense and antisense orientations. The vector pCGN3892 contains the soybean 7S promoter and a pea *rbc*S 3'. Both gene fusions are then separately ligated into pCGN9372, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter. The resulting expression constructs (pCGN5469 sense and pCGN5471 antisense) are used for transformation of soybean.

The *FAD2-1B* intron (SEQ ID NO: 2) is fused to the 3' end of the *FAD2-1A* intron in plasmid pCGN5468 (contains the soybean 7S promoter fused to the *FAD2-1A* intron (sense) and a pea *rbc*S 3') or pCGN5470 (contains the soybean 7S promoter fused to the *FAD2-1A* intron (antisense) and a pea *rbc*S 3') in sense or antisense orientation respectively. The resulting intron combination fusions are then ligated separately into pCGN9372, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter. The resulting expression constructs (pCGN5485, *FAD2-1A & FAD2-1B* intron sense and pCGN5486, *FAD2-1A & FAD2-1B* intron antisense) are used for transformation of soybean.

1B. FAD3-1 Constructs

FAD3-1A introns #1, #2, #4 and #5 (SEQ ID NOs: 7, 8, 10 and 11, respectively), *FAD3-1B* introns #3C (SEQ ID NO: 23) and #4 (SEQ ID NO: 24), are all ligated separately into pCGN3892, in sense or antisense orientations. pCGN3892 contains the soybean 7S promoter and a pea *rbc*S 3'. These fusions are ligated into pCGN9372, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter for transformation into soybean. The resulting expression constructs (pCGN5455, *FAD3-1A* intron #4 sense; pCGN5459, *FAD3-1A* intron #4 antisense; pCGN5456, *FAD3* intron #5 sense; pCGN5460, *FAD3-1A* intron #5 antisense; pCGN5466, *FAD3-1A* intron #2 antisense; pCGN5473, *FAD3-1A* intron #1 antisense) are used for transformation of soybean.

1C. FatB Constructs

The soybean *FATB* intron II sequence (SEQ ID NO: 30) is amplified via PCR using a *FATB* partial genomic clone as a template. PCR amplification is carried out as follows: 1 cycle, 95°C for 10 min; 25 cycles, 95°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec; 1 cycle, 72°C for 7 min. PCR amplification results in a product that is 854 bp long, including reengineered restriction sites at both ends. The PCR product is cloned directly into the expression cassette pCGN3892 in sense orientation, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers, to form pMON70674. Vector pCGN3892 contains the soybean 7S promoter and a pea rbcS 3'. pMON70674 is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter. The resulting gene expression construct, pMON70678, is used for transformation of soybean using *Agrobacterium* methods.

Two other expression constructs containing the soybean *FATB* intron II sequence (SEQ ID NO: 30) are created. pMON70674 is cut with *Not*I and ligated into pMON70675 which contains the CP4 *EPSPS* gene regulated by the FMV promoter and the *KAS IV* gene regulated by the napin promoter, resulting in pMON70680. The expression vector pMON70680 is then cut with *Sna*BI and ligated with a gene fusion of the jojoba delta-9 desaturase gene (SEQ ID NO: 41) in sense orientation regulated by the 7S promoter. The expression constructs pMON70680 and pMON70681 are used for transformation of soybean using *Agrobacterium* methods.

1D. Combination Constructs

Expression constructs are made containing various permutations of a first set of DNA sequences. The first set of DNA sequences are any of those described, or illustrated in FIGS. 5 and 6, or any other set of DNA sequences that contain either various combinations of sense and antisense *FAD2*, *FAD3*, and *FATB* non-coding regions so that they are capable of forming dsRNA constructs, sense cosuppression constructs, antisense constructs, or various combinations of the foregoing.

FIGS. 5(a)-(c) depict several first sets of DNA sequences which are capable of expressing sense cosuppression or antisense constructs according to the present invention, the non-coding sequences of which are described in Tables 1 and 2 below. The non-coding sequences may be single sequences, combinations of sequences (*e.g.*, the 5'UTR linked to the 3'UTR), or any combination of the foregoing. To express a sense cosuppression construct, all of the non-coding sequences are sense sequences, and to express an antisense construct, all of the non-coding sequences are antisense sequences. FIG. 5(d) depicts a first set of DNA sequences which is capable of expressing sense and antisense constructs according to the present invention.

FIGS. 6(a)-(c) depict several first sets of DNA sequences which are capable of expressing dsRNA constructs according to the present invention, the non-coding sequences of which are described in Tables 1 and 2 below. The first set of DNA sequences depicted in FIG. 6 comprises pairs of related sense and antisense sequences, arranged such that, *e.g.*, the RNA expressed by the first sense sequence is capable of forming a double-stranded RNA with the antisense RNA expressed by the first antisense sequence. For example, referring to FIG. 6(a) and illustrative combination No. 1 (of Table 1), the first set of DNA sequences comprises a sense *FAD2-1* sequence, a sense *FAD3-1* sequence, an antisense *FAD2-1* sequence and an antisense *FAD3-1* sequence. Both antisense sequences correspond to the sense sequences so that the expression products of the first set of DNA sequences are capable of forming a double-stranded RNA with each other. The sense sequences may be separated from the antisense sequences by a spacer sequence, preferably one that promotes the formation of a dsRNA molecule. Examples of such spacer sequences include those set forth in Wesley *et al.*, *supra*, and Hamilton *et al.*, *Plant J.*, *15*:737-746 (1988). The promoter is any promoter functional in a

plant, or any plant promoter. Non-limiting examples of suitable promoters are described in Part D of the Detailed Description.

The first set of DNA sequences is inserted in an expression construct in either the sense or anti-sense orientation using a variety of DNA manipulation techniques. If convenient restriction sites are present in the DNA sequences, they are inserted into the expression construct by digesting with the restriction endonucleases and ligation into the construct that has been digested at one or more of the available cloning sites. If convenient restriction sites are not available in the DNA sequences, the DNA of either the construct or the DNA sequences is modified in a variety of ways to facilitate cloning of the DNA sequences into the construct. Examples of methods to modify the DNA include by PCR, synthetic linker or adapter ligation, in vitro site-directed mutagenesis, filling in or cutting back of overhanging 5' or 3' ends, and the like. These and other methods of manipulating DNA are well known to those of ordinary skill in the art.

Table 1

Illustrative Combinations	Non-Coding Sequences (sense or antisense)			
	First	Second	Third	Fourth
1	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*		
2	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*		
3	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence	
4	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	*FATB*	
5	*FAD2-1A* or *B*	*FATB*	*FAD3-1A* or *B* or *C*	
6	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*	*FATB*	
7	*FAD3-1A* or *B* or *C*	*FATB*	*FAD2-1A* or *B*	
8	*FATB*	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*	
9	*FATB*	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	
10	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence	*FATB*
11	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*	different *FAD3-1A* or *B* or *C* sequence	*FATB*
12	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence	*FAD2-1A* or *B*	*FATB*
13	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	*FATB*	different *FAD3-1A* or *B* or *C* sequence
14	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*	*FATB*	different *FAD3-1A* or *B* or *C* sequence
15	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence	*FATB*	*FAD2-1A* or *B*
16	*FAD2-1A* or *B*	*FATB*	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence
17	*FAD3-1A* or *B* or *C*	*FATB*	*FAD2-1A* or *B*	different *FAD3-1A* or *B* or *C* sequence
18	*FAD3-1A* or *B* or *C*	*FATB*	different *FAD3-1A*	*FAD2-1A* or *B*

			or *B* or *C* sequence	
19	*FATB*	*FAD2-1A* or *B*	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence
20	*FATB*	*FAD3-1A* or *B* or *C*	*FAD2-1A* or *B*	different *FAD3-1A* or *B* or *C* sequence
21	*FATB*	*FAD3-1A* or *B* or *C*	different *FAD3-1A* or *B* or *C* sequence	*FAD2-1A* or *B*

Table 2

	Correlation of SEQ ID NOs with Sequences in Table 1					
	FAD2-1A	*FAD2-1B*	*FAD3-1A*	*FAD3-1B*	*FAD3-1C*	*FATB*
3'UTR	SEQ NO: 5	n/a	SEQ NO: 16	SEQ NO: 26	n/a	SEQ NO: 36
5'UTR	SEQ NO: 6	n/a	SEQ NO: 17	SEQ NO: 27	n/a	SEQ NO: 37
5'+3' UTR (or 3'+5' UTR)	Linked SEQ NOs: 5 and 6	n/a	Linked SEQ NOs: 16 and 17	Linked SEQ NOs: 26 and 27	n/a	Linked SEQ NOs: 36 and 37
Intron #1	SEQ NO: 1	SEQ NO: 2	SEQ NO: 7	SEQ NO: 19	n/a	SEQ NO: 29
Intron #2	n/a	n/a	SEQ NO: 8	SEQ NO: 20	n/a	SEQ NO: 30
Intron #3	n/a	n/a	n/a	n/a	n/a	SEQ NO: 31
Intron #3A	n/a	n/a	SEQ NO: 9	SEQ NO: 21	n/a	n/a
Intron #3B	n/a	n/a	SEQ NO: 12	SEQ NO: 22	n/a	n/a
Intron #3C	n/a	n/a	SEQ NO: 13	SEQ NO: 23	n/a	n/a
Intron #4	n/a	n/a	SEQ NO: 10	SEQ NO: 24	SEQ NO: 14	SEQ NO: 32
Intron #5	n/a	n/a	SEQ NO: 11	SEQ NO: 25	n/a	SEQ NO: 33
Intron #6	n/a	n/a	n/a	n/a	n/a	SEQ NO: 34
Intron #7	n/a	n/a	n/a	n/a	n/a	SEQ NO: 35

Example 2 Combination Constructs

In Figures 7-15, promoters are indicated by arrows, encoding sequences (both coding and non-coding) are indicated by pentagons which point in the direction of transcription, sense sequences are labeled in normal text, and antisense sequences are labeled in upside-down text. The abbreviations used in these Figures include: 7Sa = 7Sα promoter; 7Sa' = 7Sα' promoter; Br napin = *Brassica* napin promoter; FMV = an FMV promoter; ARC = arcelin promoter; RBC E9 3' = Rubisco E9 termination signal; Nos 3' = *nos* termination signal; TML 3' = *tml* termination signal; napin 3' = napin termination signal; '3 (in the same box as FAD or FAT) = 3' UTR; 5' (in the same box as FAD or FAT) = 5'UTR; Cr = *Cuphea pulcherrima*; Gm = Glycine max; Rc = *Ricinus communis*; FAB2 = a FAB2 allele of a stearoyl-desaturase gene; and Intr or Int = intron.

2A. dsRNA Constructs

FIGS. 7-9 depict nucleic acid molecules of the present invention in which the first sets of DNA sequences are capable of expressing dsRNA constructs. The first set of DNA sequences depicted in FIGS. 7-9 comprise pairs of related sense and antisense sequences, arranged such that, *e.g.*, the RNA expressed by the first sense sequence is capable of forming a

double-stranded RNA with the antisense RNA expressed by the first antisense sequence. The sense sequences may be adjacent to the antisense sequences, or separated from the antisense sequences by a spacer sequence, as shown in FIG. 9.

The second set of DNA sequences comprises coding sequences, each of which is a DNA sequence that encodes a sequence that when expressed is capable of increasing one or both of the protein and transcript encoded by a gene selected from the group consisting of *KAS I*, *KAS IV*, delta-9 desaturase, and CP4 *EPSPS*. Each coding sequence is associated with a promoter, which can be any promoter functional in a plant, or any plant promoter, and may be an FMV promoter, a napin promoter, a 7S (either 7Sα or 7Sα') promoter, an arcelin promoter, a delta-9 desaturase promoter, or a *FAD2-1A* promoter.

Referring now to FIG. 7, soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. Vectors containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence, and a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence, are cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON68539, is depicted in FIG. 7 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* intron 4 (SEQ ID NO: 10), and *FATB* intron II (SEQ ID NO: 30) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4

EPSPS gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON68540, is depicted in FIG. 7 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* intron 4 (SEQ ID NO: 10), and *FATB* intron II (SEQ ID NO: 30) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON68544, is depicted in FIG. 7 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* intron 4 (SEQ ID NO: 10), *FATB* intron II (SEQ ID NO: 30), and *FAD3-1B* intron 4 (SEQ ID NO: 24) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON68546, is depicted in FIG. 7 and is used for transformation using methods as described herein.

Referring now to FIG. 8, soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα'

promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON68536, is depicted in FIG. 8 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. A vector containing a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence, is cut with appropriate restriction enzymes, and ligated just upstream of the *tml* 3' termination sequence. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON68537, is depicted in FIG. 8 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON68538, is depicted in FIG. 8 and is used for transformation using methods as described herein.

Referring now to FIG. 9, soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FAD3-1B* 3'UTR (SEQ ID NO: 26) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80622, is depicted in FIG. 9 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, separated by a spliceable soybean *FAD3-1A* intron 5 (SEQ ID NO: 11), into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80623, is depicted in FIG. 9 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together), *FAD3-1A* 3'UTR (SEQ ID NO: 16) and *FAD3-1B* 5'UTR-3'UTR (SEQ ID NOs: 27 and 26, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, O5, is depicted in FIG. 9 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together) and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense and antisense orientations, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O6, is depicted in FIG. 9 and is used for transformation using methods as described herein.

2B. Sense Cosuppression Constructs

FIGS. 10-13 depict nucleic acid molecules of the present invention in which the first sets of DNA sequences are capable of expressing sense cosuppression constructs. The second set of DNA sequences comprises coding sequences, each of which is a DNA sequence that encodes a sequence that when expressed is capable of increasing one or both of the protein and transcript encoded by a gene selected from the group consisting of *KAS I*, *KAS IV*, delta-9 desaturase, and CP4 *EPSPS*. Each coding sequence is associated with a promoter, which is any promoter functional in a plant, or any plant promoter, and may be an FMV promoter, a napin promoter, a 7S promoter (either 7Sα or 7Sα'), an arcelin promoter, a delta-9 desaturase promoter, or a *FAD2-1A* promoter.

Referring now to FIG. 10, soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1C* intron 4 (SEQ ID NO: 14), *FATB* intron II (SEQ ID NO: 30), *FAD3-1A* intron 4 (SEQ ID NO: 10), and *FAD3-1B* intron 4 (SEQ ID NO: 24) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a pea Rubisco E9 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3'

termination sequence. The resulting gene expression construct, pMON68522, is depicted in FIG. 10 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* intron 4 (SEQ ID NO: 10), *FAD3-1B* intron 4 (SEQ ID NO: 24), and *FATB* intron II (SEQ ID NO: 30) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. Vectors containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence, and a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence, are cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80614, is depicted in FIG. 10 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON68531, is depicted in FIG. 10 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4

EPSPS gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. Vectors containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence, and a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence, are cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON68534, is depicted in FIG. 10 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence, is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON68535, is depicted in FIG. 10 and is used for transformation using methods as described herein.

Referring now to FIG. 11, soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80605, is depicted in FIG. 11 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in

sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80606, is depicted in FIG. 11 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80607, is depicted in FIG. 11 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FATB* 3'UTR (SEQ ID NO: 36) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. Vectors containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence, and a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated by a soybean *FAD2* promoter and a *nos* 3'

termination sequence, are cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80614, is depicted in FIG. 11 and is used for transformation using methods as described herein.

Referring now to FIG. 12, soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80629, is depicted in FIG. 12 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2), *FAD3-1A* intron 4 (SEQ ID NO: 10), *FATB* intron II (SEQ ID NO: 30), and *FAD3-1A* intron 4 (SEQ ID NO: 10) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON81902, is depicted in FIG. 12 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FAD3-1* 5'UTR-3'UTR (SEQ ID NOs: 17 and 16, ligated together, or 27 and 26, ligated together), and *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The *FAD2-1* PCR product is cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. Similarly, the *FAD3-1* PCR product is cloned directly, in sense orientation, into a vector containing the soybean 7Sα promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The *FATB* PCR product is cloned directly, in sense orientation, into a vector containing the arcelin

promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. These vectors are then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, O1, is depicted in FIG. 12 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FAD3-1* 5'UTR-3'UTR (SEQ ID NOs: 17 and 16, ligated together, or 27 and 26, ligated together), and *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The *FAD2-1* PCR product is cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. Similarly, the *FAD3-1* PCR product is cloned directly, in sense orientation, into a vector containing the soybean 7Sα promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The *FATB* PCR product is cloned directly, in sense orientation, into a vector containing the arcelin promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. These vectors are then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O2, is depicted in FIG. 12 and is used for transformation using methods as described herein.

Referring now to FIG. 13, soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FAD3-1B* 5'UTR-3'UTR (SEQ ID NOs: 27 and 26, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vectors are then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the

FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O7, is depicted in FIG. 13 and is used for transformation using methods as described herein.

Soybean *FAD2-1* intron 1 (SEQ ID NO: 1 or 2) is amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. Soybean *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FAD3-1B* 5'UTR-3'UTR (SEQ ID NOs: 27 and 26, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in sense orientation, into a vector containing the soybean 7Sα promoter and a *nos* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vectors are then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O9, is depicted in FIG. 13 and is used for transformation using methods as described herein.

2C. Antisense Constructs

FIG. 14 depicts nucleic acid molecules of the present invention in which the first sets of DNA sequences are capable of expressing antisense constructs, and FIG. 15 depicts nucleic acid molecules of the present invention in which the first sets of DNA sequences are capable of expressing combinations of sense and antisense constructs. The second set of DNA sequences comprises coding sequences, each of which is a DNA sequence that encodes a sequence that when expressed is capable of increasing one or both of the protein and transcript encoded by a gene selected from the group consisting of *KAS I*, *KAS IV*, delta-9 desaturase, and CP4 *EPSPS*. Each coding sequence is associated with a promoter, which is any promoter functional in a

plant, or any plant promoter, and may be an FMV promoter, a napin promoter, a 7S (either 7Sα or 7Sα') promoter, an arcelin promoter, a delta-9 desaturase promoter, or a *FAD2-1A* promoter.

Referring now to FIG. 14, soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in antisense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80615, is depicted in FIG. 14 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in antisense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80616, is depicted in FIG. 14 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in antisense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *R. communis* delta-9 desaturase (*FAB2*) gene (SEQ ID NO: 40) regulated

by a soybean *FAD2* promoter and a *nos* 3' termination sequence, is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, pMON80617, is depicted in FIG. 14 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 3'UTR (SEQ ID NO: 5), *FATB* 3'UTR (SEQ ID NO: 36), and *FAD3-1A* 3'UTR (SEQ ID NO: 16) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in antisense orientation, into a vector containing the soybean 7Sα promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, pMON80630, is depicted in FIG. 14 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together), *FAD3-1A* 3'UTR (SEQ ID NO: 16), and *FAD3-1B* 5'UTR-3'UTR (SEQ ID NOs: 27 and 26, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly, in antisense orientation, into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O8, is depicted in FIG. 14 and is used for transformation using methods as described herein.

Referring now to FIG. 15, soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FAD3-1A* 5'UTR-3'UTR (SEQ ID NOs: 17 and 16, ligated together), and *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly in sense and antisense orientation into a vector containing the

soybean 7Sα' promoter and a *tml* 3' termination sequence, with an additional soybean 7Sα promoter located between the sense and antisense sequences, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. The resulting gene expression construct, O3, is depicted in FIG. 15 and is used for transformation using methods as described herein.

Soybean *FAD2-1* 5'UTR-3'UTR (SEQ ID NOs: 6 and 5, ligated together), *FAD3-1A* 5'UTR-3'UTR (SEQ ID NOs: 27 and 26, ligated together), and *FATB* 5'UTR-3'UTR (SEQ ID NOs: 37 and 36, ligated together) sequences are amplified via PCR to result in PCR products that include reengineered restriction sites at both ends. The PCR products are cloned directly in sense and antisense orientation into a vector containing the soybean 7Sα' promoter and a *tml* 3' termination sequence, with an additional soybean 7Sα promoter located between the sense and antisense sequences, by way of *Xho*I sites engineered onto the 5' ends of the PCR primers. The vector is then cut with *Not*I and ligated into pMON41164, a vector that contains the CP4 *EPSPS* gene regulated by the FMV promoter and a pea Rubisco E9 3' termination sequence. A vector containing a *C. pulcherrima* KAS IV gene (SEQ ID NO: 39) regulated by a *Brassica* napin promoter and a *Brassica* napin 3' termination sequence is cut with appropriate restriction enzymes, and ligated into pMON41164. The resulting gene expression construct, O4, is depicted in FIG. 15 and is used for transformation using methods as described herein.

The above-described nucleic acid molecules are preferred embodiments which achieve the objects, features and advantages of the present invention. It is not intended that the present invention be limited to the illustrated embodiments. The arrangement of the sequences in the first and second sets of DNA sequences within the nucleic acid molecule is not limited to the illustrated and described arrangements, and may be altered in any manner suitable for achieving the objects, features and advantages of the present invention as described herein, illustrated in the accompanying drawings, and encompassed within the claims.

Example 3 Plant Transformation and Analysis

The constructs of Examples 1 and 2 are stably introduced into soybean (for example, Asgrow variety A4922 or Asgrow variety A3244 or Asgrow variety A3525) by the methods described earlier, including the methods of McCabe *et al.*, *Bio/Technology*, 6:923-926 (1988),

or *Agrobacterium*-mediated transformation. Transformed soybean plants are identified by selection on media containing glyphosate. Fatty acid compositions are analyzed from seed of soybean lines transformed with the constructs using gas chromatography. In addition, any of the constructs may contain other sequences of interest, as well as different combinations of promoters.

For some applications, modified fatty acid compositions are detected in developing seeds, whereas in other instances, such as for analysis of oil profile, detection of fatty acid modifications occurring later in the FAS pathway, or for detection of minor modifications to the fatty acid composition, analysis of fatty acid or oil from mature seeds is preferred. Furthermore, analysis of oil and/or fatty acid content of individual seeds may be desirable, especially in detection of oil modification in the segregating R1 seed populations. As used herein, R0 indicates the plant and seed arising from transformation/regeneration protocols described herein, and R1 indicates plants and seeds generated from the transgenic R0 seed.

Fatty acid compositions are determined for the seed of soybean lines transformed with the constructs of Example 2. One to ten seeds of each of the transgenic and control soybean lines are ground individually using a tissue homogenizer (Pro Scientific) for oil extraction. Oil from ground soybean seed is extracted overnight in 1.5 ml heptane containing triheptadecanoin (0.50 mg/ml). Aliquots of 200 µl of the extracted oil are derivatized to methyl esters with the addition of 500 µl sodium methoxide in absolute methanol. The derivatization reaction is allowed to progress for 20 minutes at 50°C. The reaction is stopped by the simultaneous addition of 500 µl 10% (w/v) sodium chloride and 400 µl heptane. The resulting fatty acid methyl esters extracted in hexane are resolved by gas chromatography (GC) on a Hewlett-Packard model 6890 GC (Palo Alto, CA). The GC was fitted with a Supelcowax 250 column (30 m, 0.25 mm id, 0.25 micron film thickness) (Supelco, Bellefonte, PA). Column temperature is 175°C at injection and the temperature programmed from 175°C to 245°C to 175°C at 40°C/min. Injector and detector temperatures are 250°C and 270°C, respectively.

Example 4 Synthesized Fuel Oil with Improved Biodiesel Properties

A synthesized fuel oil fatty acid composition is prepared having the following mixtures of fatty acid methyl esters: 73.3% oleic acid, 21.4% linoleic acid, 2.2% palmitic acid, 2.1% linolenic acid and 1.0% stearic acid (all by weight). Purified fatty acid methyl esters are

obtained from Nu-Chek Prep, Inc., Elysian, MN, USA. The cetane number and ignition delay of this composition is determined by the Southwest Research Institute using an Ignition Quality Tester ("IQT") 613 (Southwest Research Institute, San Antonio, Texas, USA).

An IQT consists of a constant volume combustion chamber that is electrically heated, a fuel injection system, and a computer that is used to control the experiment, record the data and provide interpretation of the data. The fuel injection system includes a fuel injector nozzle that forms an entrance to the combustion chamber. A needle lift sensor in the fuel injector nozzle detects fuel flow into the combustion chamber. A pressure transducer attached to the combustion chamber measures cylinder pressure, the pressure within the combustion chamber. The basic concept of an IQT is measurement of the time from the start of fuel injection into the combustion chamber to the start of combustion. The thermodynamic conditions in the combustion chamber are precisely controlled to provide consistent measurement of the ignition delay time.

For a cetane number and ignition delay test, the test fuel is filtered using a 5-micron filter. The fuel reservoir, injection line, and nozzle are purged with pressurized nitrogen. The fuel reservoir is then cleaned with a lint free cloth. A portion of the test fuel is used to flush the fuel reservoir, injection line, and nozzle. The reservoir is filled with the test fuel and all air is bled from the system. The reservoir is pressurized to 50 psig. The method basically consists of injecting at high pressure a precisely metered quantity of the test fuel into the combustion chamber that is charged with air to the desired pressure and temperature. The measurement consists of determining the time from the start of injection to the onset of combustion, often referred to as the ignition delay time. This determination is based on the measured needle lift and combustion chamber pressures. The normal cetane rating procedure calls for setting the skin temperature at 567.5°C. and the air pressure at 2.1 MPa.

A fuel with a known injection delay is run in the IQT combustion bomb at the beginning of the day to make sure the unit is operating within normal parameters. The test synthetic is then run. The known fuel is run again to verify that the system has not changed. Once the fuel reservoir is reconnected to the fuel injection pump, the test procedure is initiated on the PC controller. The computer controls all the procedure, including the air charging, fuel injection, and exhaust events. 32 repeat combustion events are undertaken.

The ignition delay is the time from the start of injection to the start of ignition. It is determined from the needle lift and cylinder pressure data. The rise of the injection needle signals start of injection. The cylinder pressure drops slightly due to the cooling effect of the vaporization of the fuel. Start of combustion is defined as the recovery time of the cylinder pressure – increases due to combustion to the pressure it was just prior to fuel injection.

The measured ignition delay times are then used to determine the cetane number based on a calibration curve that is incorporated into the data acquisition and reduction software. The calibration curve, consisting of cetane number as a function of ignition delay time, is generated using blends of the primary reference fuels and NEG check fuels. In the case of test fuels that are liquid at ambient conditions, the calibration curve is checked on a daily basis using at least one check fuel of known cetane number (Ryan, "Correlation of Physical and Chemical Ignition Delay to Cetane Number", SAE Paper 852103 (1985); Ryan, "Diesel Fuel Ignition Quality as Determined in a Constant Volume Combustion Bomb", SAE Paper 870586 (1986); Ryan, "Development of a Portable Fuel Cetane Quality Monitor", Belvoir Fuels and Lubricants Research Facility Report No. 277, May (1992); Ryan, "Engine and Constant Volume Bomb Studies of Diesel Ignition and Combustion", SAE Paper 881616 (1988); and Allard *et al.*, "Diesel Fuel Ignition Quality as Determined in the Ignition Quality Tester ("IQT")", SAE Paper 961182 (1996)). As shown in Table 3, the synthesized oil composition exhibits cetane numbers and ignition delays that are suitable for use for example, without limitation, as a biodiesel oil.

TABLE 3

Fuel Name	Test Number	Cetane Number	Std.Dev. Cetane No.	Ignition Delay (ms)	Std.Dev. Ign. Delay
Check-High[1]	1777	49.55	0.534	4.009	0.044
Check-High	1778	49.33	0.611	4.028	0.051
	Average	49.4		4.02	
Synthesized Oil	1779	55.02	1.897	3.622	0.116
Synthesized Oil	1780	55.65	1.807	3.583	0.109
Synthesized Oil	1781	55.63	1.649	3.583	0.098
	Average	55.4		3.60	
Check-High	1786	49.2	0.727	4.04	0.061

[1] The fuel called "Check-High" is a calibration fuel. It should have a cetane number of 49.3±0.5. The unit is checked with the calibration before and after running the synthetic test fuel.

The density (ASTM D-4052) cloud point (ASTM D-2500), pour point (ASTM D-97), and cold filter plugging point (IP 309/ASTM D-6371) are determined for the synthesized oil using ASTM D protocols. ASTM D protocols are obtained from ASTM, 100 Barr Harbor Drive, West Conshohocken, PA, USA. The results of these tests are set forth in Table 4. As shown in Table 4, the synthesized oil composition exhibits numbers that are suitable for use as, for example without limitation, as a biodiesel oil.

TABLE 4

TEST	METHOD	RESULTS
Density	ASTM D-4052	0.8791 g/mL
Cloud Point	ASTM D-2500	-18 deg. C
Pour Point	ASTM D-97	-21 deg. C
Cold Filter Plugging Point	IP 309 (same as ASTM D-6371)	-21 deg. C

Levels of nitric oxide emissions are estimated by evaluating the unsaturation levels of a biologically-based fuel, by measuring the fuel density and indirectly calculating the estimated emissions levels, or by directly measuring . There are also standard protocols available for directly measuring levels of nitric oxide emissions. The synthesized oil is estimated to have lower nitric oxide emissions levels than methyl esters of fatty acids made from conventional soybean oil based on an evaluation of the overall level of unsaturation in the synthesized oil. Oils containing larger numbers of double bonds, *i.e.*, having a higher degree of unsaturation, tend to produce higher nitric oxide emissions. The oil has a total of 123 double bonds, as compared to conventional soybean oil's total of 153 double bonds, as shown in Table 5.

TABLE 5

SYNTHETIC OIL		
73 % oleic acid (18:1)	x 1 double bond =	73
22 % linoleic acid (18:2)	x 2 double bonds =	44
2 % linolenic acid (18:3)	x 3 double bonds =	6
	TOTAL double bonds	123
CONVENTIONAL SOYBEAN OIL		
23 % oleic acid (18:1)	x 1 double bond =	23
53 % linoleic acid (18:2)	x 2 double bonds =	106
8 % linolenic acid (18:3)	x 3 double bonds =	24
	TOTAL double bonds	153

As reported by the National Renewable Energy Laboratory, Contract No. ACG-8-17106-02 Final Report, *The Effect Of Biodiesel Composition On Engine Emissions From A*

DDC Series 60 Diesel Engine, (June 2000), nitric acid emissions of biodiesel compositions are predicted by the formula y = 46.959x - 36.388 where y is the oxide emissions in grams/brake horse power hours; and x is the density of biodiesel. The formula is based on a regression analysis of nitric acid emission data in a test involving 16 biodiesel fuels. The test makes use of a 1991 calibration, production series 60 model Detroit Diesel Corporation engine.

The density of the synthesized oil is determined by Southwest Research Institute using the method ASTM D4052. The result shown in Table 4 is used in the above equation to predict a nitric oxide emission value of 4.89 g/bhp-h. This result is compared to a control soybean product. The National Renewable Energy Laboratory report gives the density and nitric oxide emissions of a control soy based biodiesel (methyl soy ester IGT). The density of the control biodiesel is 0.8877 g/mL, giving a calculated nitric oxide emission of 5.30 g/bhp-h. This calculated emission value is similar to the experimental value for nitric oxide emission of 5.32 g/bhp-h. The synthesized oil composition exhibits improved numbers compared to the control and is suitable for use, for example without limitation, as a biodiesel oil.

Example 5 Optimum Fatty Acid Composition For Healthy Serum Lipid Levels

The cholesterol lowering properties of vegetable compositions are determined to identify fatty acid compositions that have a more favorable effect on serum lipid levels than conventional soybean oil (*i.e.*, lower LDL-cholesterol and higher HDL-cholesterol). Published equations based on 27 clinical trials (Mensink, R.P. and Katan, M.B. *Arteriosclerosis and Thrombosis*, 12:911-919 (1992)) are used to compare the effects on serum lipid levels in humans of new oilseed compositions with that of normal soybean oil.

Table 6 below presents the results of the change in serum lipid levels where 30% of dietary energy from carbohydrate is substituted by lipids. The results show that soybean oil already has favorable effects on serum lipids when it replaces carbohydrates in the diet. Improvements on this composition are possible by lowering saturated fat levels and by obtaining a linoleic acid level between 10-30% of the total fatty acids, preferably about 15-25% of the total fatty acids. When the proportion of linoleic acid is less than 10% of the total fatty acids, the new composition raises LDL-cholesterol compared to control soybean oil, even though the saturated fat content is lowered to 5% of the total fatty acids. When the proportion of linoleic acid is increased, the ability of the composition to raise serum HDL levels is

reduced. Therefore, the preferred linoleic acid composition is determined to be about 15-25% of the total fatty acids.

Table 6

	Fatty acids						Serum Lipids
	C16:0	C18:0	C18:1	C18:2	C18:3	Other (C20:1)	
Soy control (%)	11.000	4.000	23.400	53.200	7.800	0.600	
Proportion of 30% fat E (%)	3.300	1.200	7.020	15.960	2.340	0.180	
LDL Calculation (mg/dl)	4.224	1.536	1.685	8.778	1.287	0.043	-6.033
HDL Calc (mg/dl)	1.551	0.564	2.387	4.469	0.655	0.061	9.687
3% 18:2, <6% sat (%)	3.000	2.000	85.000	3.000	3.000	4.000	
Proportion of 30% fat E (%)	0.900	0.600	25.500	0.900	0.900	1.200	
LDL Calculation (mg/dl)	1.152	0.768	6.120	0.495	0.495	0.288	-5.478
vs. control (mg/dl)							**0.555**
HDL calculation (mg/dl)	0.423	0.282	8.670	0.252	0.252	0.408	10.287
vs. control (mg/dl)							**0.600**
10% 18:2, <6% sat (%)	3.000	2.000	72.000	10.000	3.000	10.000	
Proportion of 30% fat E (%)	0.900	0.600	21.600	3.000	0.900	3.000	
LDL Calculation (mg/dl)	1.152	0.768	5.184	1.650	0.495	0.720	-6.129
vs. control (mg/dl)							**-0.096**
HDL calculation (mg/dl)	0.423	0.282	7.344	0.840	0.252	1.020	10.161
vs. control (mg/dl)							**0.474**
20% 18:2, <6% sat (%)	3.000	2.000	65.000	20.000	3.000	7.000	
Proportion of 30% fat E (%)	0.900	0.600	19.500	6.000	0.900	2.100	
LDL Calculation (mg/dl)	1.152	0.768	4.680	3.300	0.495	0.504	-7.059
vs. control (mg/dl)							**-1.026**
HDL calculation (mg/dl)	0.423	0.282	6.630	1.680	0.252	0.714	9.981
vs. control (mg/dl)							**0.294**
21% 18:2, <3.2% sat (%)	2.000	1.000	72.000	21.000	1.000	3.000	
Proportion of 30% fat E (%)	0.600	0.300	21.600	6.300	0.300	0.900	
LDL Calculation (mg/dl)	0.768	0.384	5.184	3.465	0.165	0.216	-7.878
vs. control (mg/dl)							**-1.845**
HDL calculation (mg/dl)	0.282	0.141	7.344	1.764	0.084	0.306	9.921
vs. control (mg/dl)							**0.234**
30% 18:2, <6% sat (%)	3.000	2.000	57.000	30.000	3.000	5.000	
Proportion of 30% fat E (%)	0.900	0.600	17.100	9.000	0.900	1.500	
LDL Calculation (mg/dl)	1.152	0.768	4.104	4.950	0.495	0.360	-7.989
vs. control (mg/dl)							**-1.956**
HDL calculation (mg/dl)	0.423	0.282	5.814	2.520	0.252	0.510	9.801
vs. control (mg/dl)							**0.114**

What is claimed is:

1. A soybean seed exhibiting an oil composition comprising 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

2. The soybean seed of claim 1, wherein said seed comprises a recombinant nucleic acid molecule, said molecule comprising

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least two genes selected from the group consisting of FAD2, FAD3, and FATB genes, and

a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

3. The soybean seed of claim 2, wherein said seed exhibits an increased oleic acid content, a reduced saturated fatty acid content, and a reduced polyunsaturated fatty acid content relative to seed from a plant with a similar genetic background but lacking the recombinant nucleic acid molecule.

4. The soybean seed of claim 2, wherein the oil composition further comprises 10 to 39% by weight linoleic acid, 4.5% or less by weight linolenic acid, and 3 to 6% by weight saturated fatty acids.

5. The soybean seed of claim 2, wherein the oil composition further comprises 10 to 39% by weight linoleic acid, 3.0% or less by weight linolenic acid, and 2 to 3.6% by weight saturated fatty acids.

6. The soybean seed of claim 2, wherein the oil composition further comprises 11 to 30% by weight linoleic acid, 4.5% or less by weight linolenic acid, and less than 6% by weight saturated fatty acids.

7. Oil derived from the soybean seed of claim 2, wherein said oil exhibits an increased oleic acid content, a reduced saturated fatty acid content, and a reduced polyunsaturated fatty acid content relative to oil derived from seed of a plant with a similar genetic background but lacking the recombinant nucleic acid molecule.

8. Meal derived from the soybean seed of claim 2.

9. A container of soybean seeds, wherein at least 25% of the seeds exhibit an oil composition comprising 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

10. A soybean seed exhibiting an oil composition comprising 65 to 80% by weight oleic acid, 10 to 30% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

11. The soybean seed of claim 10, wherein the oil composition further comprises 10 to 29% by weight linoleic acid, 4.5% or less by weight linolenic acid, and 3 to 6% by weight saturated fatty acids.

12. The soybean seed of claim 10, wherein the oil composition further comprises 10 to 29% by weight linoleic acid, 3.0% or less by weight linolenic acid, and 2 to 3.6% by weight saturated fatty acids.

13. A crude soybean oil exhibiting an oil composition comprising 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

14. The crude soybean oil of claim 13, wherein said oil is selected from the group consisting of a cooking oil, a salad oil, and a frying oil.

15. The crude soybean oil of claim 13, wherein said oil is a raw material for making a substance selected from the group consisting of shortening, margarine, lubricant, biodiesel, heating oil, and diesel additive.

16. The crude soybean oil of claim 13, wherein said oil is produced in a volume greater than one liter.

17. The crude soybean oil of claim 16, wherein said oil is produced in a volume greater than ten liters.

18. A crude soybean oil exhibiting an oil composition comprising 65 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

19. A crude soybean oil exhibiting an oil composition which comprises 69 to 73% by weight oleic acid, 21 to 24% by weight linoleic acid, 0.5 to 3% by weight linolenic acid, and 2-3% by weight of saturated fatty acids.

20. The crude soybean oil of claim 19, wherein said oil is selected from the group consisting of a cooking oil, a salad oil, and a frying oil.

21. The crude soybean oil of claim 19, wherein said oil is a raw material for making a soyfood.

22. A transformed soybean plant bearing seed, wherein said seed exhibits an oil composition which comprises 55 to 80% by weight oleic acid, 10 to 40% by weight linoleic acid, 6% or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

23. The transformed soybean plant of claim 22, wherein said transformed soybean plant comprises a recombinant nucleic acid molecule which comprises

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of a FAD2 gene and a FAD3 gene, and

a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

24. Feedstock derived from the transformed plant of claim 23.

25. A plant part derived from the transformed plant of claim 23.

26. Seed derived from the transformed plant of claim 23.

27. A transformed plant comprising a recombinant nucleic acid molecule which comprises

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least two genes selected from the group consisting of FAD2, FAD3, and FATB genes, and

a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

28. The transformed plant of claim 27, wherein said transformed plant is a temperate oilseed plant.

29. The transformed plant of claim 27, wherein said transformed plant is a soybean plant.

30. The transformed plant of claim 27, wherein said transformed plant produces a seed with an increased oleic acid content, a reduced saturated fatty acid content, and a reduced polyunsaturated fatty acid content, relative to a plant with a similar genetic background but lacking the recombinant nucleic acid molecule.

31. A method of altering the oil composition of a plant cell comprising:

(A) transforming a plant cell with a recombinant nucleic acid molecule which comprises a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least two genes selected from the group consisting of FAD2, FAD3, and FATB genes, and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene; and

(B) growing said plant cell under conditions wherein transcription of said first set of DNA sequences and said second set of DNA sequences is initiated, whereby said oil composition is altered relative to a plant cell with a similar genetic background but lacking the recombinant nucleic acid molecule.

32. The method of claim 31, wherein said growing step produces a plant cell with at least partially reduced levels of a FAD2 enzyme and a FAD3 enzyme, and at least partially enhanced levels of said at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

33. The method of claim 31, wherein said cell is present in a multicellular environment.

34. The method of claim 33, wherein said cell is present in a transformed plant.

35. The method of claim 31, wherein said alteration comprises an increased oleic acid content, a reduced saturated fatty acid content, and a reduced polyunsaturated fatty acid content, relative to a plant cell with a similar genetic background but lacking the recombinant nucleic acid molecule.

36. A method of producing a transformed plant having seed with a reduced saturated fatty acid content comprising:

(A) transforming a plant cell with a recombinant nucleic acid molecule which comprises a first set of DNA sequences that is capable, when expressed in a host cell, of

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suppressing the endogenous expression of at least two genes selected from the group consisting of FAD2, FAD3, and FATB genes, and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene; and

(B) growing the transformed plant, wherein the transformed plant produces seed with a reduced saturated fatty acid content relative to seed from a plant having a similar genetic background but lacking the recombinant nucleic acid molecule.

37. The method of claim 36, wherein said growing step further comprises expressing the first set of DNA sequences and said second set of DNA sequences in a tissue or organ of a plant, wherein said tissue or organ is selected from the group consisting of roots, tubers, stems, leaves, stalks, fruit, berries, nuts, bark, pods, seeds and flowers.

38. The method of claim 36, wherein said growing step further comprises expressing the first set of DNA sequences and said second set of DNA sequences in a seed.

39. A recombinant nucleic acid molecule comprising:

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of at least two genes selected from the group consisting of FAD2, FAD3, and FATB genes; and

a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

40. The recombinant nucleic acid molecule of claim 39, wherein said first set of DNA sequences comprises a first non-coding sequence that is capable, when expressed in a host cell, of suppressing the endogenous expression of a *FAD2* gene; and a second non-coding sequence that is capable, when expressed in a host cell, of suppressing the endogenous expression of a *FAD3-1A* gene.

41. The recombinant nucleic acid molecule of claim 40, wherein the first set of DNA sequences is expressed as a sense cosuppression RNA transcript.

42. The recombinant nucleic acid molecule of claim 40, wherein the first non-coding sequence is expressed as a first sense cosuppression RNA transcript, and the second non-coding sequence is expressed as a second sense cosuppression RNA transcript, and the first and second sense cosuppression transcripts are not linked to each other.

43. The recombinant nucleic acid molecule of claim 40, wherein the first set of DNA sequences is expressed as an antisense RNA transcript.

44. The recombinant nucleic acid molecule of claim 40, wherein the first non-coding sequence is expressed as a first antisense RNA transcript, and the second non-coding sequence is expressed as a second antisense RNA transcript, and the first and second antisense transcripts are not linked to each other.

45. The recombinant nucleic acid molecule of claim 40, wherein the first set of DNA sequences is expressed as an RNA transcript capable of forming a single double-stranded RNA molecule.

46. The recombinant nucleic acid molecule of claim 40, wherein said first set of DNA sequences further comprises a third non-coding sequence that is capable, when expressed in a host cell, of suppressing the endogenous expression of a *FAD3-1B* gene.

47. The recombinant nucleic acid molecule of claim 46, wherein said first non-coding sequence is a *FAD2-1A* sequence, said second non-coding sequence is a *FAD3-1A* sequence, and said third non-coding sequence is a *FAD3-1B* sequence.

48. The recombinant nucleic acid molecule of claim 47, wherein said *FAD2-1A* sequence is selected from the group consisting of a *FAD2-1A* intron sequence, a *FAD2-1A* 3'UTR sequence, and a *FAD2-1A* 5'UTR sequence.

49. The recombinant nucleic acid molecule of claim 47, wherein said *FAD3-1A* sequence is selected from the group consisting of a *FAD3-1A* intron sequence, a *FAD3-1A* 3' UTR sequence, and a *FAD3-1A* 5' UTR sequence.

50. The recombinant nucleic acid molecule of claim 47, wherein said *FAD3-1B* sequence is selected from the group consisting of a *FAD3-1B* intron sequence, a *FAD3-1B* 3'UTR sequence, and a *FAD3-1B* 5'UTR sequence.

51. The recombinant nucleic acid molecule of claim 40, wherein said first set of DNA sequences further comprises a third non-coding sequence that is capable, when expressed in a host cell, of suppressing the endogenous expression of a *FATB* gene.

52. The recombinant nucleic acid molecule of claim 51, wherein said *FATB* sequence is selected from the group consisting of a *FATB* intron sequence, a *FATB* 3' UTR sequence, and a *FATB* 5' UTR sequence.

53. The recombinant nucleic acid molecule of claim 39, further comprising a plant promoter operably linked to said first set of DNA sequences.

54. The recombinant nucleic acid molecule of claim 53, wherein said plant promoter is a *FAD2-1A* promoter, a 7Sα promoter, or a 7Sα' promoter.

55. The recombinant nucleic acid molecule of claim 39, wherein said second set of DNA sequences is capable, when expressed, of increasing the endogenous expression of at least two genes selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

56. The recombinant nucleic acid molecule of claim 39, wherein said second set of DNA sequences is capable, when expressed, of increasing the endogenous expression of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

57. The recombinant nucleic acid molecule of claim 39, wherein said first set of DNA sequences and said second set of DNA sequences are arranged in a monocistronic configuration.

58. The recombinant nucleic acid molecule of claim 39, wherein said second set of DNA sequences and said second set of DNA sequences are arranged in a polycistronic configuration.

59. A recombinant nucleic acid molecule comprising:

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of a *FAD2* gene and a *FAD3* gene, wherein said first set of DNA sequences comprises a first non-coding sequence that expresses a first RNA sequence that exhibits at least 90% identity to a non-coding region of a *FAD2* gene, a first antisense sequence that expresses a first antisense RNA sequence capable of forming a double-stranded RNA molecule with the first RNA sequence, a second non-coding sequence that expresses a second RNA sequence that exhibits at least 90% identity to a non-coding region of a *FAD3* gene, and a second antisense sequence that expresses a second antisense RNA sequence capable of forming a double-stranded RNA molecule with the second RNA sequence;

and a second set of DNA sequences that is capable, when expressed in a host cell, of increasing the endogenous expression of at least one gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

60. The recombinant nucleic acid molecule of claim 59, wherein said non-coding region of a *FAD2* gene is selected from the group consisting of a *FAD2-1A* intron sequence, a *FAD2-1A* 3'UTR sequence, and a *FAD2-1A* 5'UTR sequence.

61. The recombinant nucleic acid molecule of claim 59, wherein said non-coding region of a *FAD3* gene is selected from the group consisting of a *FAD3-1A* intron sequence, a *FAD3-1A* 3'UTR sequence, and a *FAD3-1A* 5'UTR sequence.

62. The recombinant nucleic acid molecule of claim 59, wherein said non-coding region of a *FAD3* gene is selected from the group consisting of a *FAD3-1B* intron sequence, a *FAD3-1B* 3'UTR sequence, and a *FAD3-1B* 5'UTR sequence.

63. The recombinant nucleic acid molecule of claim 59, wherein the first set of DNA sequences is expressed as an RNA transcript capable of forming a single double-stranded RNA molecule.

64. The recombinant nucleic acid molecule of claim 59, further comprising a spacer sequence that separates the first and second non-coding sequences from the first and second antisense sequences such that the first set of DNA sequences is capable, when expressed, of forming a single double-stranded RNA molecule.

65. The recombinant nucleic acid molecule of claim 64, wherein said spacer sequence is a spliceable intron sequence.

66. The recombinant nucleic acid molecule of claim 65, wherein said spliceable intron sequence is a spliceable *FAD3* intron #5 sequence or a spliceable PDK intron sequence.

67. The recombinant nucleic acid molecule of claim 59, wherein said non-coding region of a *FAD3* gene is a *FAD3-1A* sequence, and wherein said first set of DNA sequences further comprises a third non-coding sequence that expresses a third RNA sequence that exhibits at least 90% identity to a non-coding region of a *FAD3-1B* gene, and a third antisense sequence that expresses a third antisense RNA sequence capable of forming a double-stranded RNA molecule with the third RNA sequence.

68. The recombinant nucleic acid molecule of claim 59, further comprising a third non-coding sequence that is capable of expressing a third RNA sequence that exhibits at least 90% identity to a non-coding region of a *FATB* gene, and a third antisense sequence that is capable of expressing a third antisense RNA sequence capable of forming a double-stranded RNA molecule with the third RNA sequence.

69. The recombinant nucleic acid molecule of claim 68, wherein said *FATB* sequence is selected from the group consisting of a *FATB* intron sequence, a *FATB* 3' UTR sequence, and a *FATB* 5' UTR sequence.

70. A recombinant nucleic acid molecule comprising:

a first set of DNA sequences that is capable, when expressed in a host cell, of suppressing the endogenous expression of a FAD2 gene and a FAD3 gene; and

a second set of DNA sequences that comprises a first coding sequence that is capable of expressing a CP4 EPSPS gene, and a second coding sequence that is capable, when expressed, of increasing the endogenous expression of a gene selected from the group consisting of a beta-ketoacyl-ACP synthase I gene, a beta-ketoacyl-ACP synthase IV gene, and a delta-9 desaturase gene.

71. The recombinant nucleic acid molecule of claim 70, wherein said first set of DNA sequences and said second set of DNA sequences are located on a single T-DNA region.

72. The recombinant nucleic acid molecule of claim 70, wherein said first set of DNA sequences and said second coding sequence are located on a first T-DNA region, and said first coding sequence is located on a second T-DNA region.

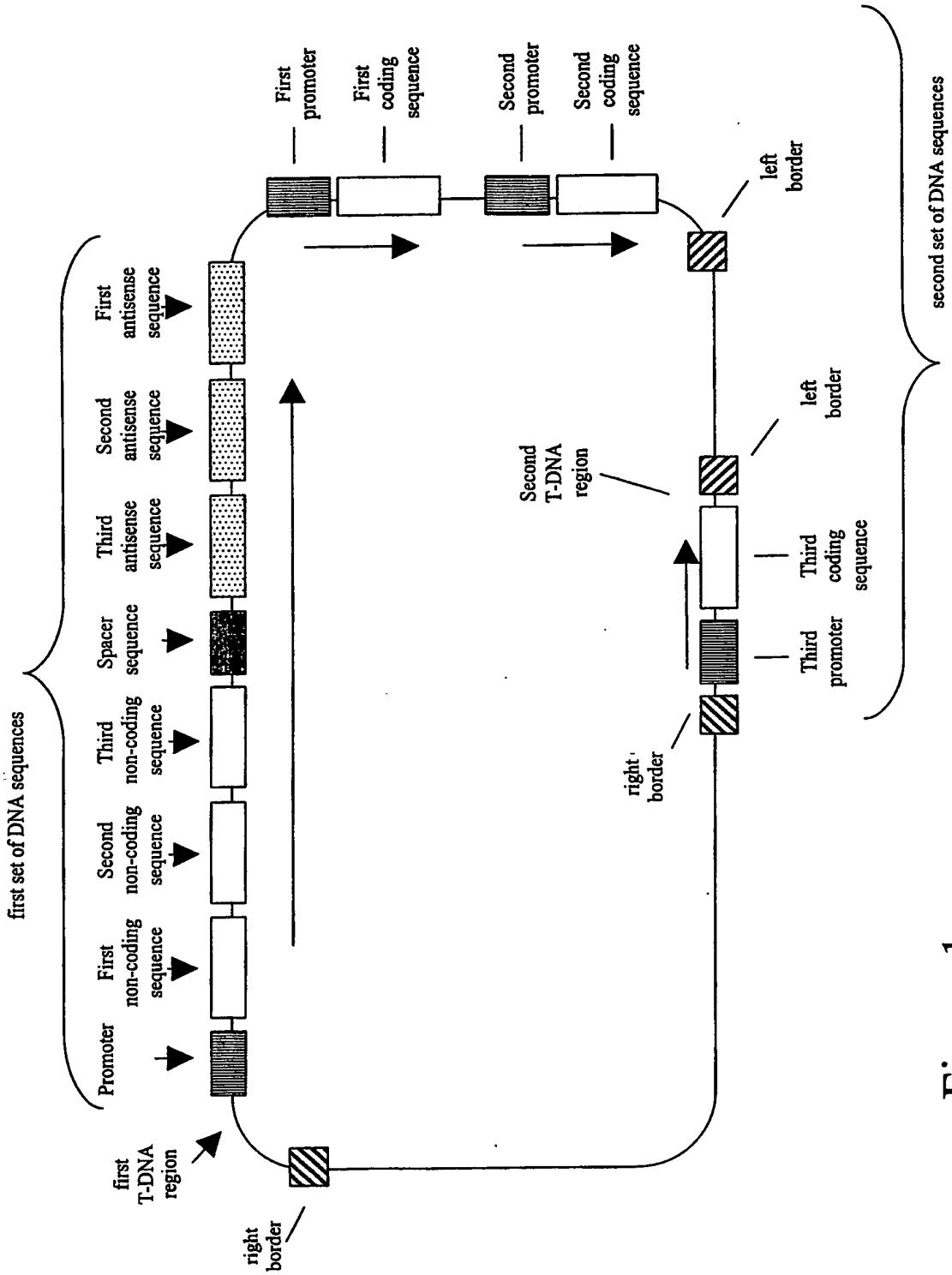


Figure 1

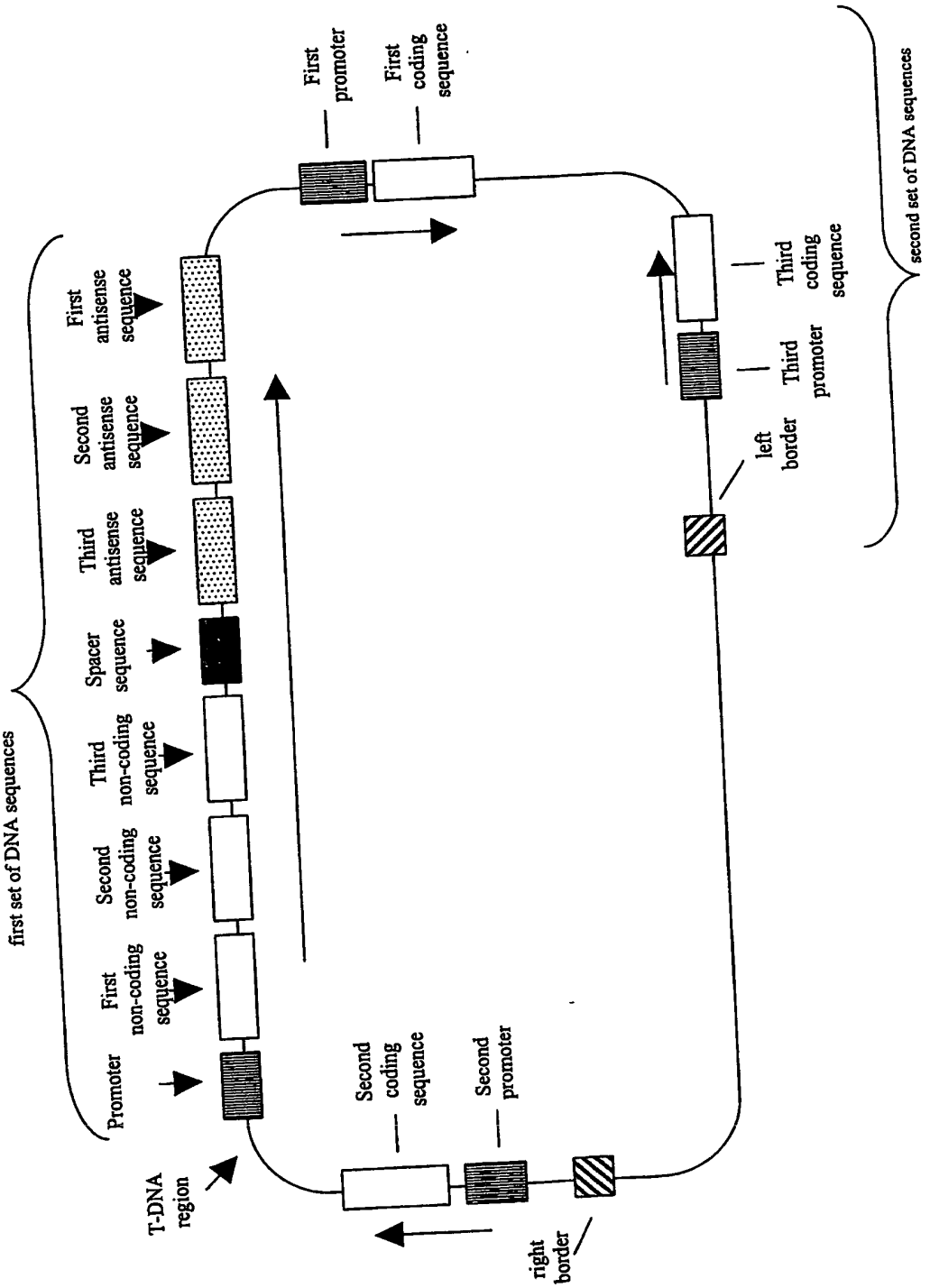

first set of DNA sequences
Promoter
First non-coding sequence
Second non-coding sequence
Third non-coding sequence
Spacer sequence
Third antisense sequence
Second antisense sequence
First antisense sequence
T-DNA region
Second coding sequence
Second promoter
right border
First promoter
First coding sequence
Third coding sequence
Third promoter
left border
second set of DNA sequences

Figure 2

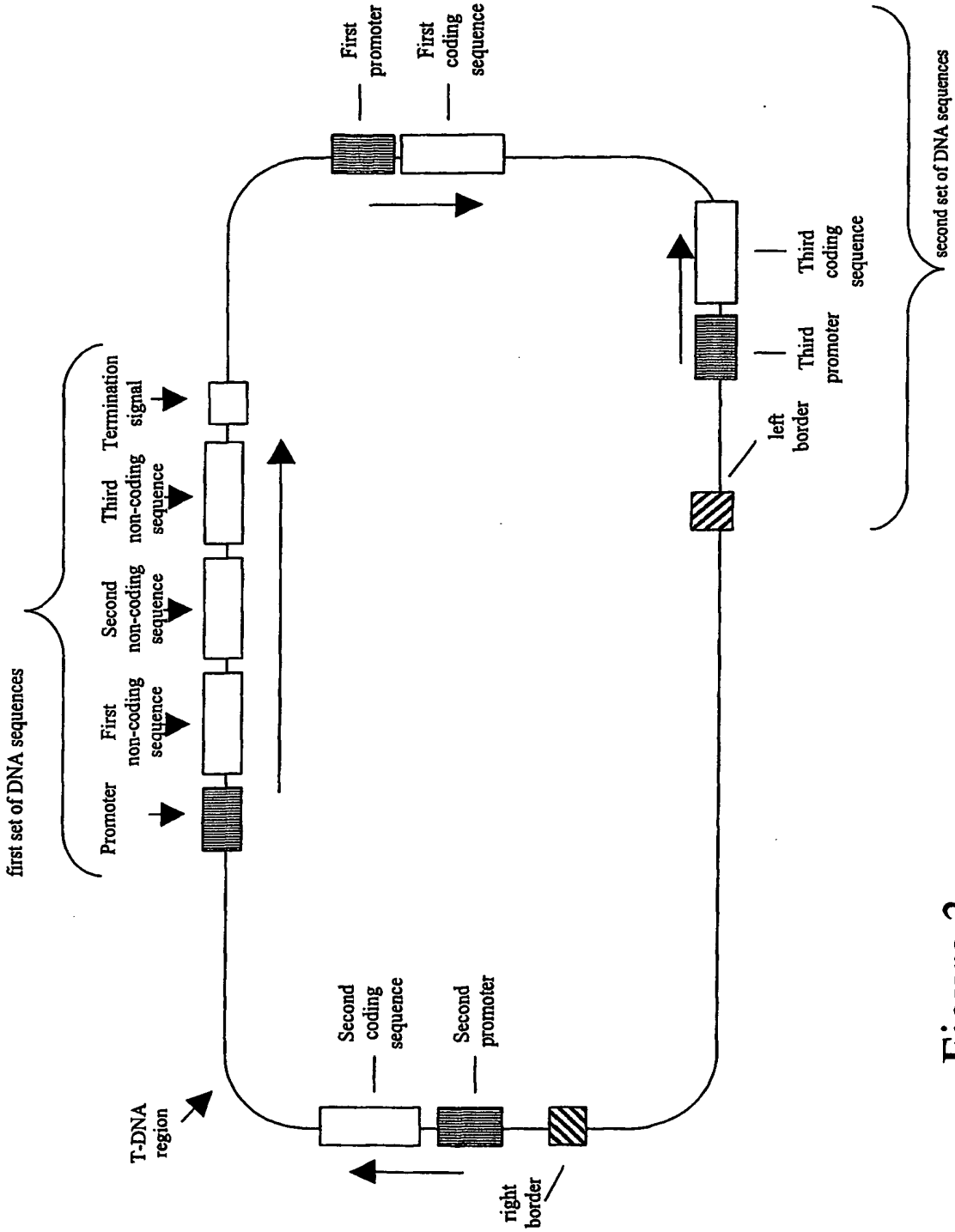


Figure 3

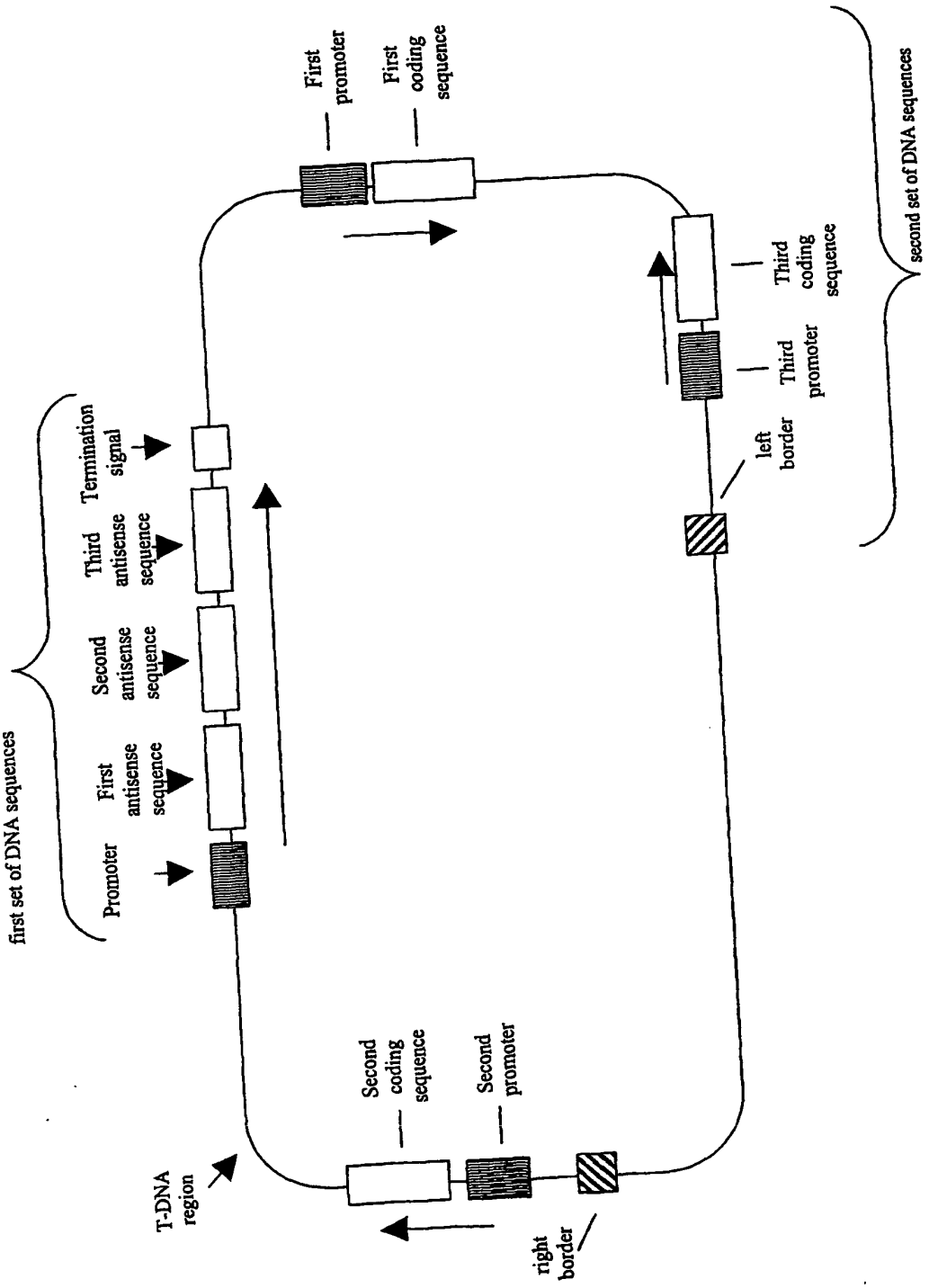

first set of DNA sequences
Promoter
First antisense sequence
Second antisense sequence
Third antisense sequence
Termination signal
T-DNA region
Second coding sequence
Second promoter
right border
left border
Third promoter
Third coding sequence
First promoter
First coding sequence
second set of DNA sequences

Figure 4

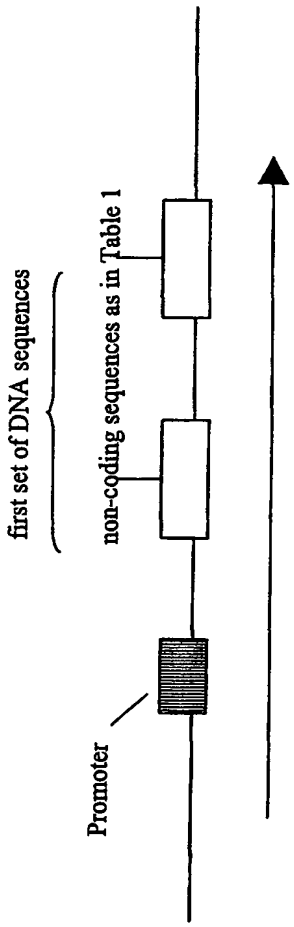


Figure 5(a)

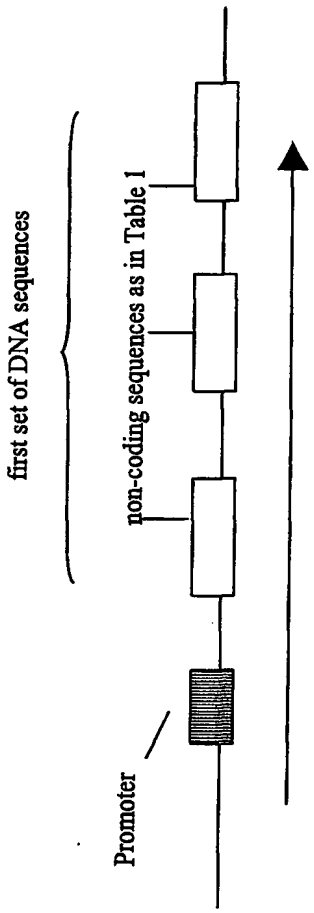


Figure 5(b)

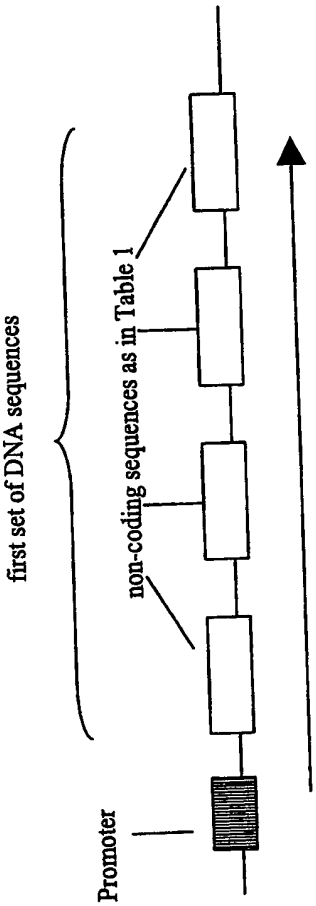


Figure 5(c)

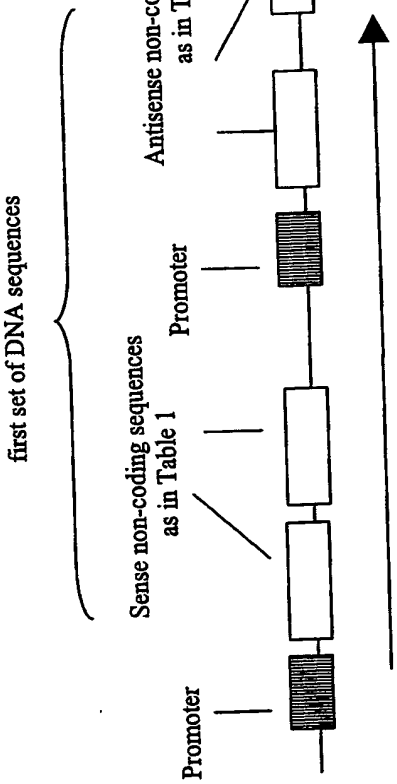


Figure 5(d)

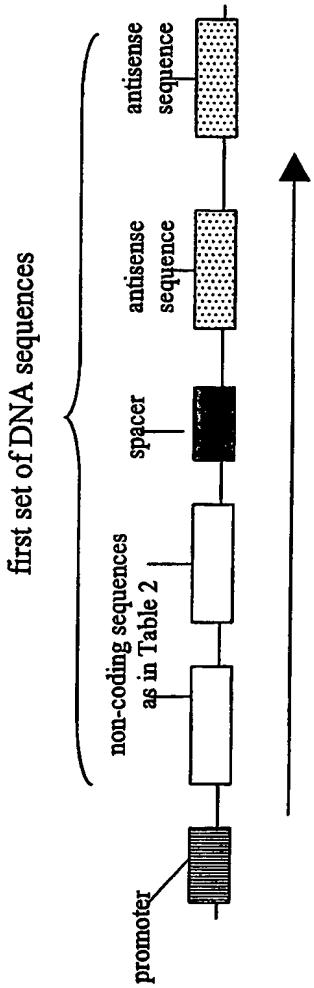


Figure 6(a)

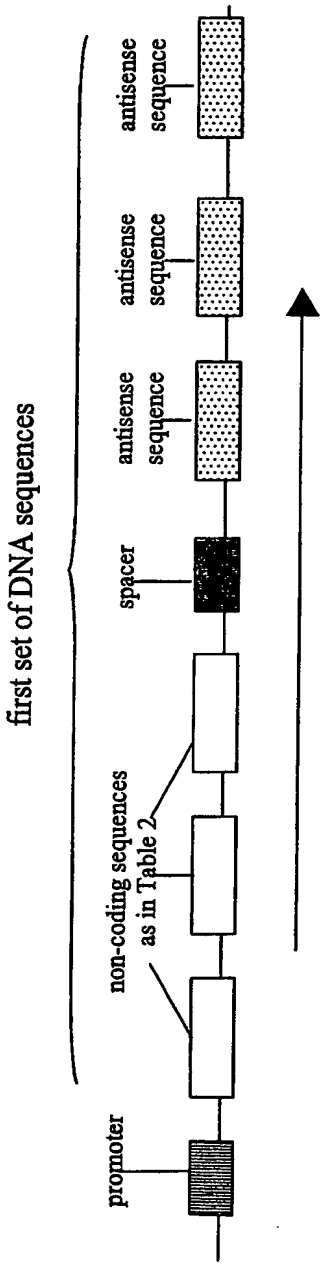


Figure 6(b)

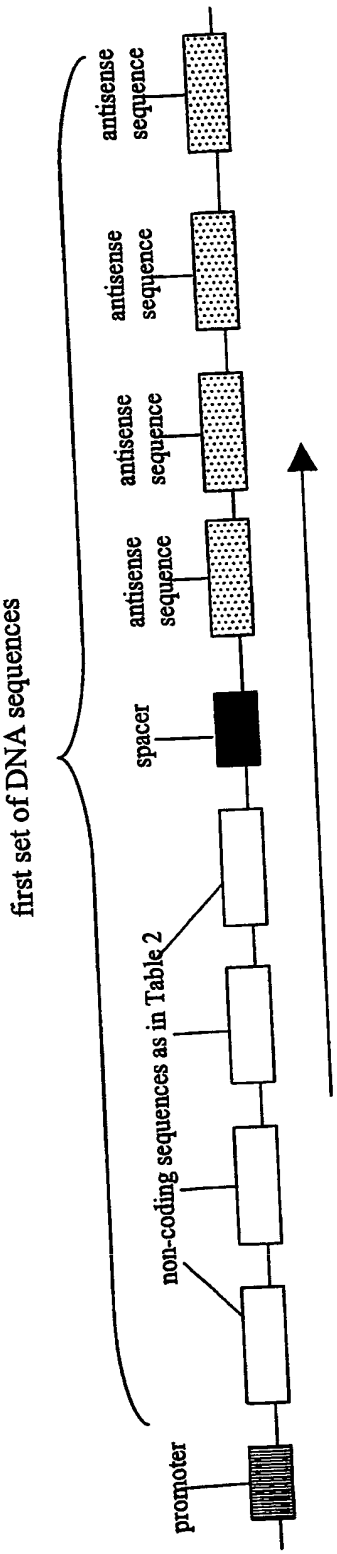

first set of DNA sequences
antisense sequence
antisense sequence
antisense sequence
antisense sequence
spacer
non-coding sequences as in Table 2
promoter

Figure 6(c)

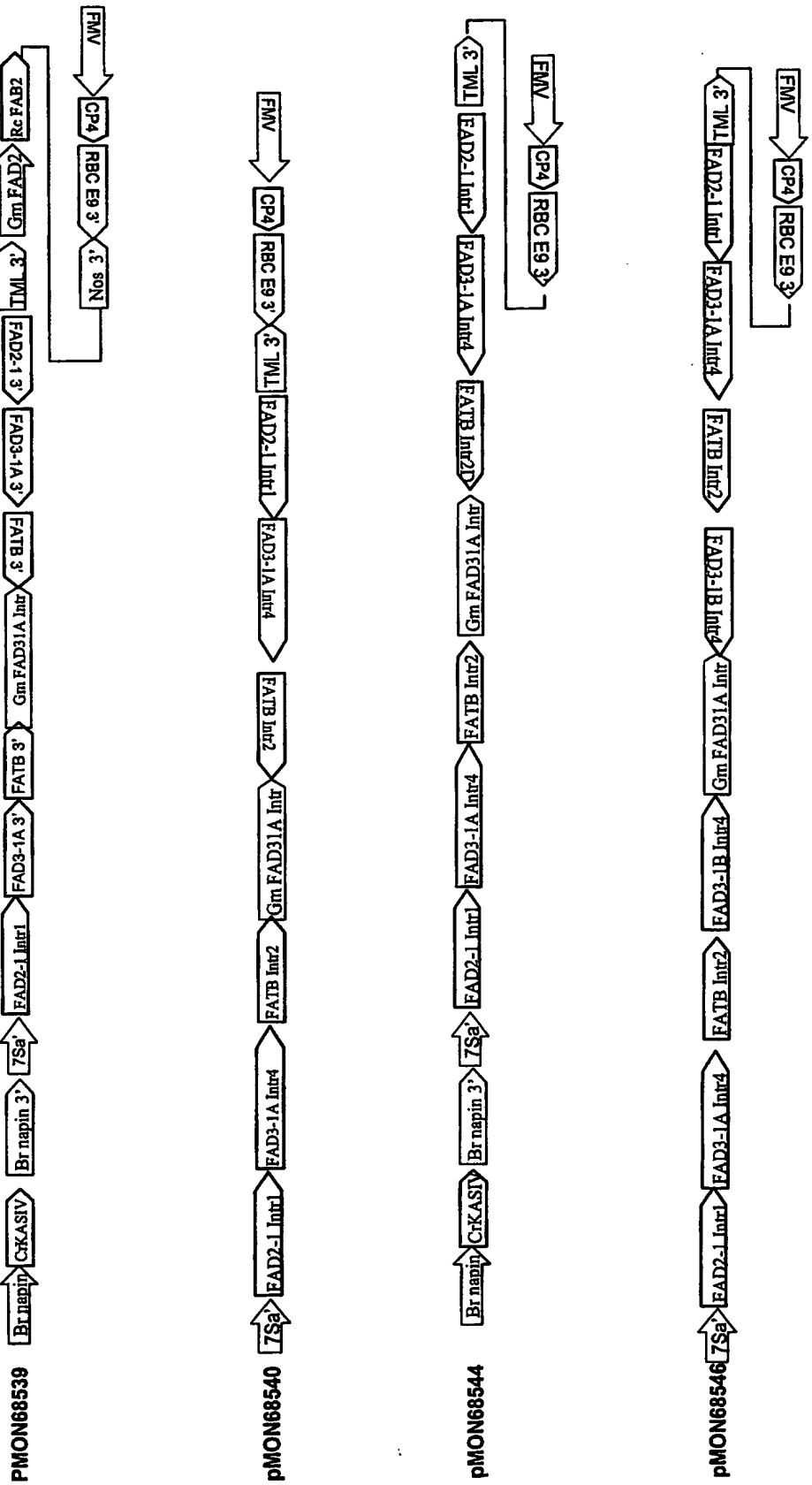


Figure 7

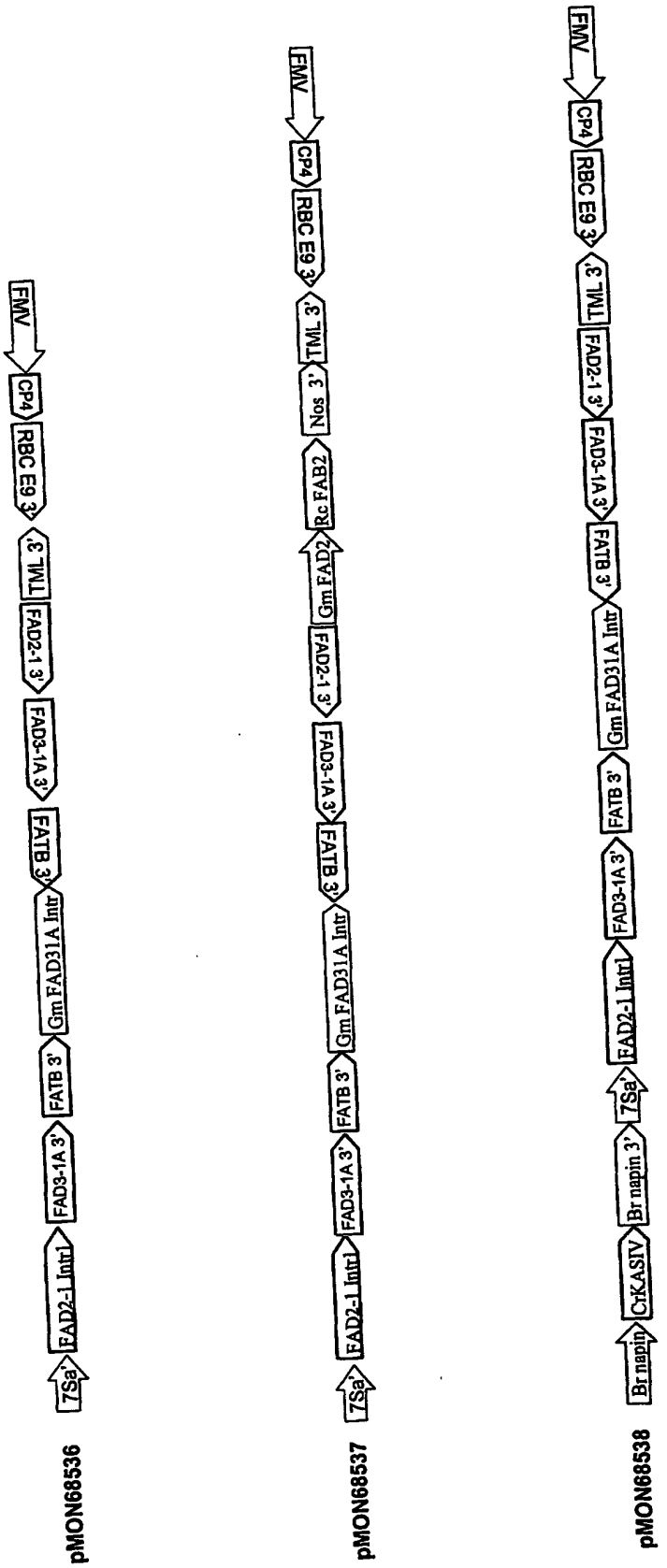

pMON68536
7Sa
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
Gm FAD31A Intr
FATB 3'
FAD3-1A 3'
FAD2-1 3'
TML 3'
RBC E9 3'
CP4
FMV
pMON68537
7Sa
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
Gm FAD31A Intr
FATB 3'
FAD3-1A 3'
FAD2-1 3'
Gm FAD2
Rc FAB2
Nos 3'
TML 3'
RBC E9 3'
CP4
FMV
pMON68538
Br napin
CrKASIV
Br napin 3'
7Sa
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
Gm FAD31A Intr
FATB 3'
FAD3-1A 3'
FAD2-1 3'
TML 3'
RBC E9 3'
CP4
FMV

Figure 8

Figure 9

PMON68522
7Sa'
FAD2-1 Intr1
FAD3-1C Intr4
FATB Intr2
FAD3-1A Intr4
FAD3-1B Intr4
RBC E9 3'
RBC E9 3'
CP4
FMV
PMON80614
Br napin
CrKASIV
Br napin 3'
7Sa'
FAD2-1 Intr1
FAD3-1A Intr4
FAD3-1B Intr4
FATB Intr2
TML 3'
Gm FAD2
Rc FAB2
Nos 3'
RBC E9 3'
CP4
FMV
pMON68531
7Sa'
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
TML 3'
RBC E9 3'
CP4
FMV
pMON68534
Br napin
CrKASIV
Br napin 3'
7Sa'
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
TML 3'
Gm FAD2
Rc FAB2
Nos 3'
RBC E9 3'
CP4
FMV
PMON68535
7Sa'
FAD2-1 Intr1
FAD3-1A 3'
FATB 3'
TML 3'
Gm FAD2
Rc FAB2
Nos 3'
RBC E9 3'
CP4
FMV

Figure 10

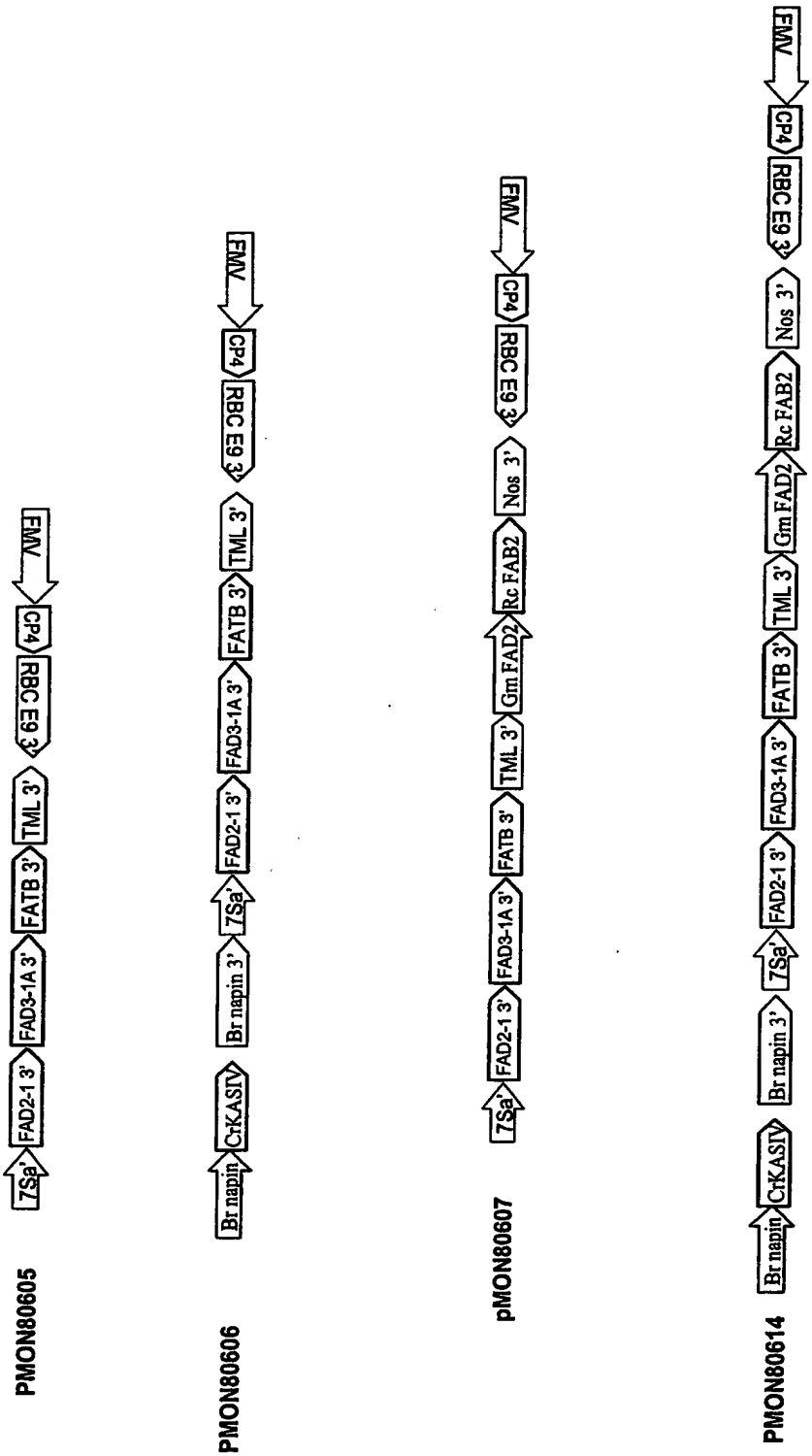


Figure 11

PMON80629
7Sa
FAD2-1 3'
FATB 3'
FAD3-1A 3'
TML 3'
RBC E9 3'
CP4
FMV
PMON81902
7Sa
FAD2-1 Intr1
FAD3-1A Intr4
FATB Intr2
FAD3-1A Intr4
TML 3'
RBC E9 3'
CP4
FMV
O1
7Sa'
FAD2-1 5+3'
TML 3'
7SA
FAD3-1 5+3'
TML 3'
ARC
FATB 5+ 3'
TML 3'
RBC E9 3'
CP4
FMV
O2
Br napin
CrKASIV
Br napin 3'
7Sa'
FAD2-1 5+3'
TML 3'
7SA
FAD3-1 5+3'
TML 3'
ARC
FATB 5+ 3'
TML 3'
RBC E9 3'
CP4
FMV

Figure 12

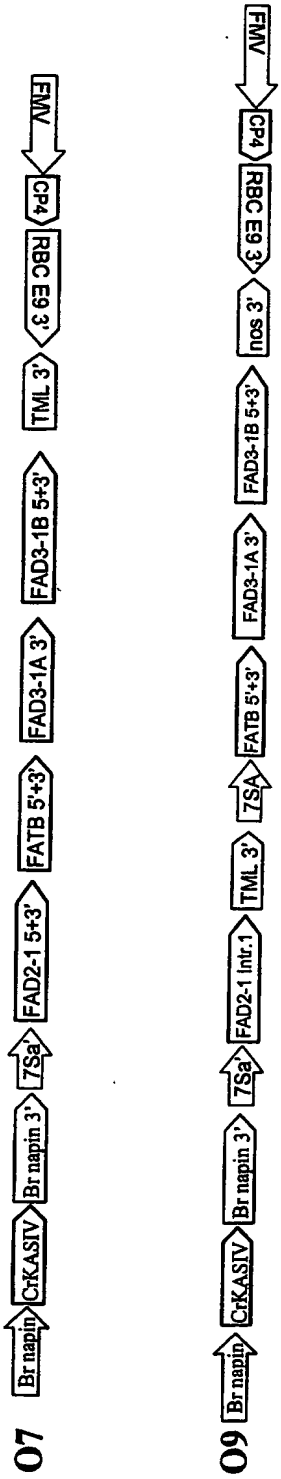
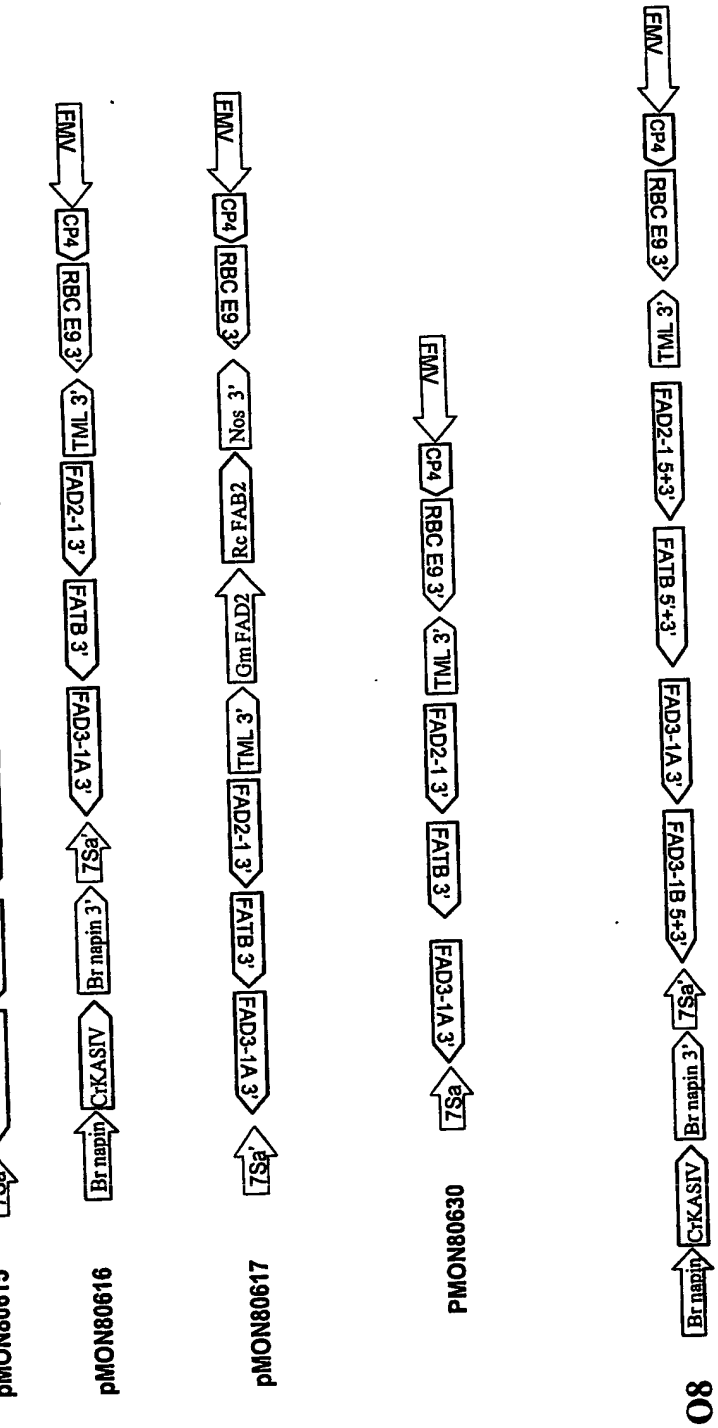


Figure 13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FMV
CP4
RBC E9 3'
TML 3'
FAD2-1 3'
FATB 3'
FAD3-1A 3'
pMON80616
FMV
CP4
RBC E9 3'
TML 3'
FAD2-1 3'
FATB 3'
FAD3-1A 3'
Br napin
CrKASIV
Br napin 3'
pMON80617
FMV
CP4
RBC E9 3'
Nos 3'
Rc FAD2
Dm FAD2
TML 3'
FAD2-1 3'
FATB 3'
FAD3-1A 3'
pMON80630
FMV
CP4
RBC E9 3'
TML 3'
FAD2-1 3'
FATB 3'
FAD3-1A 3'
pMON80808
FMV
CP4
RBC E9 3'
TML 3'
FAD2-1 5+3'
FATB 5'+3'
FAD3-1A 3'
FAD3-1B 5+3'
Br napin
CrKASIV
Br napin 3'

Figure 14

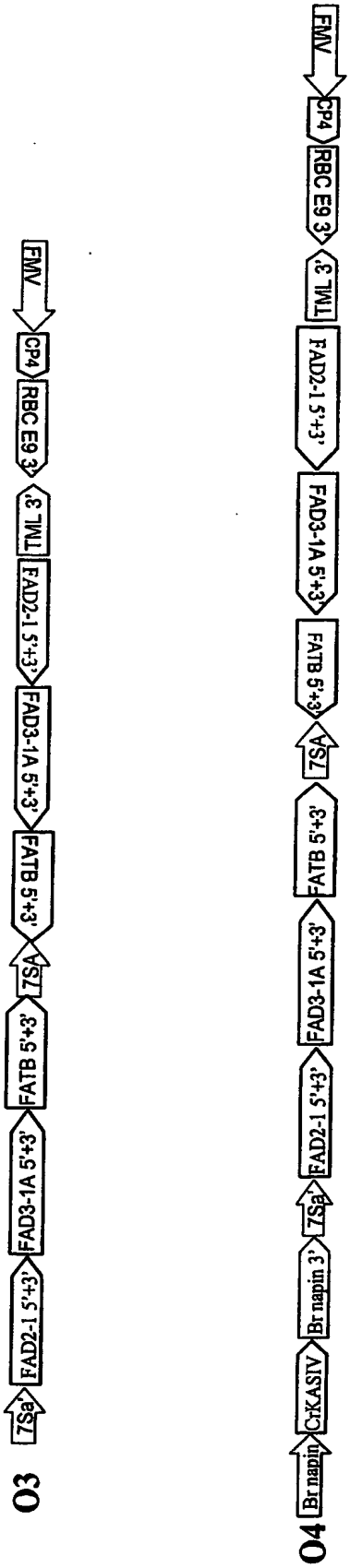

Figure 15

SEQUENCE LISTING

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<120> Nucleic Acid Constructs and Methods for Producing Altered Seed Oil Compositions

<130> 16518.098

<150> US 60/365,794
<151> 2002-03-21

<150> US 60/390,185
<151> 2002-06-21

<160> 41

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cagcttgttg cgatcatggt tataatgtaa aaataattct ggtattaatt acatgtggaa 180

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<213> Glycine max

<220>
<223> FAD3-1A 3'UTR

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agacatggaa tatttatttg aaattagtaa ggtagtaata ataaattttg aattgtcagt 180

ttca 184

<210> 17
<211> 143
<212> DNA
<213> Glycine max

<220>
<223> FAD3-1A 5'UTR

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aagcaaaagc agcagcagca gca 143

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<212> DNA
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<220>
<223> partial FAD3-1B genomic clone

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ttcataaatt atgctagaaa tgcacttact tttcaaagca tgc 2683

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<213> Glycine max

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<223> FAD3-1B intron 1

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tcagtgatgc tttagtcatt tcatttcact tggttatgca tgattgttcg ttcatatgtt 120

ctgtcatggt gagttctaat ttgattgatg catggaacag 160

<210> 20

<211> 119
<212> DNA
<213> Glycine max

<220>
<223> FAD3-1B intron 2

<400> 20
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aaaatctttg gtctgattta acaacctaac catttttaca actcatggat tttttgcag 119

<210> 21
<211> 166
<212> DNA
<213> Glycine max

<220>
<223> FAD3-1B intron 3a

<400> 21
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agtggttaga tttggttgtg ttccgataga agaaaagggg gtatctagag agatgtgaat 120

ttcatgaagt ggttcatgat tatgtgtctt tatgccttta tgtcag 166

<210> 22
<211> 156
<212> DNA
<213> Glycine max

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<223> FAD3-1B intron 3b

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tatttctaaa attttgaatt cttgaaattg aaaggaaagg actttattgg gtctagcatc 120

tcactctctc tttgtgatat gaaccatata tttcag 156

<210> 23
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<212> DNA
<213> Glycine max

<220>
<223> FAD3-1B intron 3c

<400> 23
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acaccgaagt aatgccctat aattaatgag gttaacacat gtccaagtcc aatattttgt 120

tcacttattt gaacttgaac atgtgtag 148

<210> 24
<211> 351
<212> DNA
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<220>
<223> FAD3-1B intron 4

<400> 24
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atgaccaaat tgtcctcaca agtccaaacc gtccactatt ttattttcac ctactttata 180

gcccaatttg ccatttggtt acttcagaaa agagaacccc atttgtagta aatatattat 240

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aagaaggtag aaacttactc catgttactc tgtctatatg taatttcaca g 351

<210> 25
<211> 277
<212> DNA
<213> Glycine max

<220>
<223> FAD3-1B intron 5

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atatgtcaat tgaaaattgg tcaagaccac aaacatgtga tgatctccca ccttacatat 180

aataatttct cctattctac aatcaataat ccttctatgg tcctgaattg ttcctttctt 240

ttttcatttt cttattcttt ttgttgtccc acaatag 277

<210> 26
<211> 158
<212> DNA
<213> Glycine max

<220>
<223> FAD3-1B 3'UTR

<400> 26
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tgctagaaat gcacttactt ttcaaagcat gctatgtc 158

<210> 27
<211> 83
<212> DNA

<213> Glycine max

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<223> FAD3-1B 5'UTR

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gagaaagaaa catttgagca aaa 83

<210> 28
<211> 4083
<212> DNA
<213> Glycine max

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<223> FATB genomic clone

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<220>
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<212> DNA
<213> Glycine max

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<223> FATB intron II

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<210> 31
<211> 169
<212> DNA
<213> Glycine max

<220>
<223> FATB intron III

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Published:
— with international search report

(88) Date of publication of the international search report:
12 February 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/080802 A3

(54) Title: NUCLEIC ACID CONSTRUCTS AND METHODS FOR PRODUCING ALTERED SEED OIL COMPOSITIONS

(57) Abstract: The present invention is in the field of plant genetics and provides recombinant nucleic acid molecules, constructs, and other agents associated with the coordinate manipulation of multiple genes in the fatty acid synthesis pathway. In particular, the agents of the present invention are associated with the simultaneous enhanced expression of certain genes in the fatty acid synthesis pathway and suppressed expression of certain other genes in the same pathway. Also provided are plants incorporating such agents, and in particular plants incorporating such constructs where the plants exhibit altered seed oil compositions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/08610

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A01H 5/00; 5/10
US CL : 800/312

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 800/312

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, BIOSIS AGRICOLA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,714,670 A (FEHR et al) 3 February 1998 (03.02.1998), column 25, line 42.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 October 2003 (24.10.2003)

Date of mailing of the international search report
13 NOV 2003

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Authorized officer
Elizabeth F. McElwain

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/08610

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US03/08610

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1, drawn to a non-recombinant soybean seed having comprising 55-80% by weight oleic acid, 10-40% by weight linoleic acid, 6%or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

Group II, claim(s) 1-6, drawn to a transgenic soybean seed having specified levels of fatty acids.

Group III, claim(s) 7 and 13-21, drawn to soybean oil.

Group IV, claim(s) 8, drawn to soybean meal.

Group V, claim(s) 9, drawn to a container of soybean seeds that is at least 25% comprised of soybean seeds having a specified fatty acid composition.

Group VI, claim(s) 10-12, drawn to non-recombinant soybean seed comprising 65-80% by weight oleic acid, 10-30% by weight linoleic acid, 6%or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

Group VII, claim(s) 22, 23, 25 and 26, drawn to a transformed soybean plant having a seed oil composition of 55-80% by weight oleic acid, 10-40% by weight linoleic acid, 6%or less by weight linolenic acid, and 2 to 8% by weight saturated fatty acids.

Group VIII, claim(s) 24, drawn to feedstock derived from the plant of claim 23.

Group IX, claim(s) 27-30, drawn to a transformed plant comprising two sets of DNA sequences.

Group X, claim(s) 31-35, drawn to a method of altering oil composition of a plant cell.

Group XI, claim(s) 36-38, drawn to a method of producing a transformed plant with reduced saturated fatty acid content.

Group XII, claim(s) 39, 45, 53, 54, 57 and 58, drawn to a recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least one gene.

Group XIII, claim(s) 40-42, drawn to recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least one gene wherein said sequences are noncoding regions.

Group XIV, claim(s) 40, 43 and 44, drawn to recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least one gene wherein said sequences are antisense.

Group XV, claims 46-50, drawn to recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least one gene and to a third sequence to suppress FAD3-1B.

Group XVI, claims 51 and 52, drawn to recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least one gene and to a third sequence to suppress FATB.

Group XVII, claim 55, drawn to a recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least two genes.

Group XVIII, claim 56, drawn to a recombinant nucleic acid comprising a first set of DNA sequences for suppression of at least two genes and a second set of DNA sequences for increasing expression of at least three genes.

Group XIX, claims 59-63, drawn to a recombinant nucleic acid comprising FAD2 and FAD3 genes in antisense.

Group XX, claim 64, drawn to a recombinant nucleic acid comprising FAD2 and FAD3 genes in antisense further comprising a spacer to form double stranded DNA.

Group XXI, claims 65 and 66, drawn to a recombinant nucleic acid comprising FAD2 and FAD3 genes in antisense further comprising a spacer to form double stranded DNA wherein a spliceable intron is the spacer.

Group XXII, claim 67, drawn to a recombinant nucleic acid with a third antisense sequence to FA3-1B.

Group XXIII, claims 68 and 69, drawn to a recombinant nucleic acid with a third antisense sequence to FATB.

Group XXIV, claims 70-72, drawn to a recombinant nucleic acid with a second set of DNA sequences capable of expressing a CP4 EPSPS.

The inventions listed as Groups I-XXIV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The inventions of Groups I-XXIV lack a corresponding special technical feature as evidenced by the prior art reference of U.S. Patent 5,714,670 (see column 25, line 42), which teaches a soybean seed having the fatty acid profile set forth in claim 1. Therefore, there is no special technical feature that distinguishes the claimed inventions from the prior art. In addition, the products of Groups I-IX and XII-XXIV are each chemically, structurally and functionally distinct one from each of the others; and the methods of Groups X and XI differ one from the other in that they each use different starting materials and result in different products. Furthermore, the products required in the methods of Groups X and XI differ in content and scope from the products of Groups I-IX and XXIV, and there is no corresponding special technical feature.